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show files
File 155:MEDLINE(R) 1966-2002/Aug W3
File   5:Biosis Previews(R) 1969-2002/Aug W2
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File 315:ChemEng & Biotec Abs 1970-2002/Jul
         (c) 2002 DECHEMA
File  73:EMBASE 1974-2002/Aug W3
         (c) 2002 Elsevier Science B.V.
?ds

Set     Items   Description
S1    8062370   TREAT? OR THERAP?
S2       3456   (ABNORMAL OR ABERRANT OR DEFECT?)(5N)SPLIC?
S3     775225   EXPRESS? (5N) GENE?
S4      27183   ALTERNATIVE(3N)SPLIC?
S5      57577   CYSTIC()FIBROSIS
S6     939501   MUTATION? ? OR MUTANT? ?
S7       2254   SR(2N)PROTEIN? ?
S8        229   HETEROGENEOUS(3N)NUCLEAR(3N)RIBONUCLEOPROTEIN(3N)A1
S9         10   VIRAL(3N)FACTOR? ?(3N)E4?
S10       534   HNRNP(2N)A1
S11      1355   SR()PROTEIN? ?
S12       394   S11 AND S4
S13       116   S12 AND S3
S14         9   S13 AND S1
S15         5   S13 AND (DISORDER? ? OR DISEAS? OR SYNDROME OR ILLNESS? ?)
S16       116   S2 AND S3 AND S4
S17       229   S13 OR S16
S18         0   1 S2 AND S5
S19       121   S10 AND S4
S20         3   S19 AND S5
S21        34   S19 AND S3
S22        36   S2 AND CFTR
S23        91   S9 OR S14 OR S15 OR S18 OR S20 OR S21 OR S22
S24        48   RD S23 (unique items)
S25       220   AU=KEREM B? OR AU=KEREM, B?
S26         3   S25 AND S2
S27        50   S24 OR S26
S28        48   RD S27 (unique items)
S29        29   S1 AND S2 AND S4
S30        22   RD S29 (unique items)
S31        20   S30 NOT S24
?t 31/7/all

 31/7/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13493884   22079028   PMID: 12084454
   Control of alternative splicing by antisense oligonucleotides as a
potential chemotherapy: effects on gene expression.
  Mercatante Danielle R; Kole Ryszard
  Department of Pharmacology and Lineberger Comprehensive Cancer Center,
University of North Carolina, Chapel Hill, NC 27599, USA.
  Biochimica et biophysica acta (Netherlands)  Jul 18 2002,  1587  (2-3)
 p126-32,  ISSN 0006-3002   Journal Code: 0217513
  Document type: Journal Article; Review; Review, Tutorial
  Languages: ENGLISH
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Main Citation Owner: NLM
Record type: Completed

Expression of alternatively spliced mRNA variants at specific stages of development or in specific cells and tissues contributes to the functional diversity of the human genome. Aberrations in alternative splicing were found as a cause or a contributing factor to the development, progression, or maintenance of various diseases including cancer. The use of antisense oligonucleotides to modify aberrant expression patterns of alternatively spliced mRNAs is a novel means of potentially controlling such diseases. However, to utilize antisense oligonucleotides as molecular chemotherapeutic agents, the global effects of these molecules need to be examined. The advent of gene expression array technology has now made it possible to simultaneously examine changes that occur in the expression levels of several thousand genes in response to antisense treatment . This analysis should help in the development of more specific and efficacious antisense oligonucleotides as molecular therapeutics . (94 Refs.)

Record Date Created: 20020626

31/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12903715 21818610 PMID: 11830556

A missplicedform of the cholecystokinin-B/gastrin receptor in pancreatic carcinoma: role of reduced sellular U2AF35 and a suboptimal 3'-splicing site leading to retention of the fourth intron.

Ding Wei-Qun; Kuntz Susan M; Miller Laurence J

Center for Basic Research in Digestive Diseases, Department of Biochemistry/Molecular Biology, Mayo Clinic and Foundation, 200 First Street SW, Rochester, Minnesota 55905, USA.

Cancer research (United States) Feb 1 2002, 62 (3) p947-52, ISSN 0008-5472 Journal Code: 2984705R

Contract/Grant No.: DK 32878; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Abnormal splicing of primary RNA transcripts of normal genes is a recognized mechanism for the production of some abnormal proteins found in cancer cells. A missplicedform of the cholecystokinin-B/gastrin (CCK-B) receptor recently was reported to be present in colon carcinoma, where it was postulated to play a role in stimulating tumor growth (M. R. Hellmich et al., J. Biol. Chem., 275: 32122-32128, 2000). Here, we report the presence of the same abnormal protein in pancreatic carcinoma and explore the molecular basis for this missplicing event. Reverse transcription-PCR and sequencing were used to demonstrate the presence of a missplicedform of the CCK-B receptor having its fourth intron retained in three pancreatic cancer cell lines and in tumor tissue, but not in surrounding healthy pancreas, from two patients with pancreatic carcinoma. A mini-gene construct representing the region of this gene from its third through its fifth exon and containing the two intervening introns was produced and transiently expressed in the MIA PaCa-2 human pancreatic cancer cell line. Specific reverse transcription-PCR reactions with both vector-derived and receptor-specific primers demonstrated the presence of both correctly fully spliced and selectively missplicedforms of this receptor. Mutagenesis of the mini-gene demonstrated that a suboptimal sequence at the 3'-end of intron 4 contributed to this missplicing. This focused attention on the U2

small nuclear ribonucleoprotein particle auxiliary splicing factors (U2AFs) known to interact specifically with this domain. Indeed, quantitative real-time PCR demonstrated a reduced level of expression of one of these factors, U2AF35, in pancreatic cancer cells compared with healthy pancreas. Furthermore, the relative amount of missplicing of the CCK-B receptor mini-gene in the pancreatic cancer cell line was reversed by transfection of the cells with U2AF35 cDNA. This work describes the presence of an additional abnormal protein in pancreatic cancer and describes a new molecular mechanism for its production, providing additional potential therapeutic targets.
Record Date Created: 20020206

31/7/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12615024 21560992 PMID: 11560926
Correction of alternative splicing of tau in frontotemporal dementia and parkinsonism linked to chromosome 17.
Kalbfuss B; Mabon S A; Misteli T
National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.
Journal of biological chemistry (United States) Nov 16 2001, 276 (46) p42986-93, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Mutations in the human tau gene cause frontotemporal dementia and Parkinsonism associated with chromosome 17 (FTDP-17). One of the major disease mechanisms in FTDP-17 is the increased inclusion of tau exon 10 during pre-mRNA splicing. Here we show that modified oligonucleotides directed against the tau exon 10 splice junctions suppress inclusion of tau exon 10. The effect is mediated by the formation of a stable pre-mRNA-oligonucleotide hybrid, which blocks access of the splicing machinery to the pre-mRNA. Correction of tau splicing occurs in a tau minigene system and in endogenous tau RNA in neuronal pheochromocytoma cells and is specific to exon 10 of the tau gene. Antisense oligonucleotide-mediated exclusion of exon 10 has a physiological effect by [illegible] of protein lacking the microtubule-binding domain encoded by exon 10. As a consequence, the microtubule cytoskeleton becomes destabilized and cell morphology is altered. Our results demonstrate that alternative splicing defects of tau as found in FTDP-17 patients can be corrected by application of antisense oligonucleotides. These findings provide a tool to study specific tau isoforms in vivo and might lead to a novel therapeutic strategy for FTDP-17.
Record Date Created: 20011112

31/7/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11007020 20555533 PMID: 11105947
Two genetic defects in alphaIIb are associated with type I Glanzmann's thrombasthenia in a Great Pyrenees dog: a 14-base insertion in exon 13 and a splicing defect of intron 13.
Lipscomb D L; Bourne C; Boudreaux M K

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA. lipscdl@vetmed.auburn.edu
Veterinary pathology (UNITED STATES) Nov 2000, 37 (6) p581-8, ISSN 0300-9858 Journal Code: 0312020
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Glannzmann's thrombasthenia (GT) is an autosomal recessive bleeding disorder caused by qualitative or quantitative deficiencies of the platelet membrane glycoprotein alphaIIbbeta3. This is the first report of a molecular genetic basis for type I GT in dogs. As previously reported, a thrombasthenic Great Pyrenees dog (dog No. 1) experienced uncontrolled epistaxis despite results of coagulation screening tests, platelet quantitation, and von Willebrand factor quantitation that were within reference ranges. Platelet aggregation was minimal in response to agonists. Flow cytometry, autoradiography, and immunoblot experiments demonstrated either marked reduction or absence of glycoproteins alphaIIb and beta3. In this study, we report the presence of a 14-base insertion in exon 13 and defective splicing of intron 13 in the alphaIIb gene of two thrombasthenic dogs (Nos. 1 and 8). The insertion disrupted the fourth alphaIIb calcium-binding domain, caused a shift in the reading frame and resulted in a premature termination codon. Possible consequences of this mutation include decreased alphaIIb mRNA stability and production of truncated alphaIIb protein that lacks the transmembrane and cytoplasmic domains and a large portion of the extracellular domain. We identified the dam, sire, and three littermates of dog No. 8 as carriers of the alphaIIb mutation. Canine alphaIIb and beta3 genes share significant homology with the genes in human beings, making canine GT an excellent translational model for human GT. A defined molecular basis for canine GT will enhance ongoing gene therapy research and increase the understanding of structure-function relationships of this integrin.
Record Date Created: 20010228

31/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10872482 20433583 PMID: 10979205
Repression of aberrant splicing in human beta-globin pre-mRNA with HbE mutation by antisense oligoribonucleotide or splicing factor SF2/ASF.
Shirohzu H; Yamaza H; Fukumaki Y
Division of Disease Genes, Kyushu University, Fukuoka, Japan.
International journal of hematology (IRELAND) Jul 2000, 72 (1) p28-33, ISSN 0925-5710 Journal Code: 9111627
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hemoglobin (Hb) E is the most common Hb variant among Southeast Asian populations. The mutation in codon 26 (GAG to AAG) of the beta-globin gene (beta E) induces alternative splicing , resulting in the production of normally and aberrantly spliced beta-globin mRNA. Compound heterozygosity for beta-thalassemia and HbE, beta-thalassemia/HbE disease, could lead to a severe thalassemia phenotype. Repression of aberrant splicing from the beta E mutation could ameliorate the severity in such patients. We showed that the aberrant splicing was partially repressed in cells treated

with antisense oligoribonucleotide targeted to the aberrant 5' splice site. The maximum effect of the antisense oligoribonucleotide was observed at a concentration of 0.4 mumol/L, 36 hours after the treatment in our experiment. We also analyzed the effect of the transient and stable expression of SF2/ASF on aberrant splicing in cells expressing the beta E-globin gene. Partial repression of the aberrant splicing was also observed in both expression systems. Our results imply that antisense oligoribonucleotide treatment and SF2/ASF expression are possible therapeutic applications for beta-thalassemia/HbE disease.
Record Date Created: 20001017

31/7/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10785010 20320707 PMID: 10862084
Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects .
Messiaen L M; Callens T; Mortier G; Beysen D; Vandenbroucke I; Van Roy N; Speleman F; Paepe A D
Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium. Ludwine.Messiaen@rug.ac.be
Human mutation (UNITED STATES) 2000, 15 (6) p541-55, ISSN 1059-7794 Journal Code: 9215429
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders and is caused by mutations in the NF1 gene. Mutation detection is complex due to the large size of the NF1 gene, the presence of pseudogenes and the great variety of possible lesions. Although there is no evidence for locus heterogeneity in NF1, mutation detection rates rarely exceed 50%. We studied 67 unrelated NF1 patients fulfilling the NIH diagnostic criteria, 29 familial and 38 sporadic cases, using a cascade of complementary techniques. We performed a protein truncation test starting from puromycin- treated EBV cell lines and, if no mutation was found, continued with heteroduplex, FISH, Southern blot and cytogenetic analysis. We identified the germline mutation in 64 of 67 patients and 32 of the mutations are novel. This is the highest mutation detection rate reported in a study of typical NF1 patients. All mutations were studied at the genomic and RNA level. The mutational spectrum consisted of 25 nonsense, 12 frameshift, 19 splice mutations, six missense and/or small in-frame deletions, one deletion of the entire NF1 gene, and a translocation t(14;17)(q32;q11.2). Our data suggest that exons 10a-10c and 37 are mutation-rich regions and that together with some recurrent mutations they may account for almost 30% of the mutations in classical NF1 patients. We found a high frequency of unusual splice mutations outside of the AG/GT 5 cent and 3 cent splice sites. As some of these mutations form stable transcripts, it remains possible that a truncated neurofibromin is formed.
Record Date Created: 20000811

31/7/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10643640 20171433 PMID: 10704448

Massive idiosyncratic exon skipping corrects the nonsense mutation in dystrophic mouse muscle and produces functional revertant fibers by clonal expansion.

Lu Q L; Morris G E; Wilton S D; Ly T; Artem'yeva O V; Strong P; Partridge T A

Muscle Cell Biology, Medical Research Council Clinical Science Center, Hammersmith Hospital, London W12 ONN, UK. qi.long-lu@csc.mrc.ac.uk

Journal of cell biology (UNITED STATES) Mar 6 2000, 148 (5) p985-96, ISSN 0021-9525 Journal Code: 0375356

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Conventionally, nonsense mutations within a gene preclude synthesis of a full-length functional protein. Obviation of such a blockage is seen in the mdx mouse, where despite a nonsense mutation in exon 23 of the dystrophin gene, occasional so-called revertant muscle fibers are seen to contain near-normal levels of its protein product. Here, we show that reversion of dystrophin expression in mdx mice muscle involves unprecedented massive loss of up to 30 exons. We detected several alternatively processed transcripts that could account for some of the revertant dystrophins and could not detect genomic deletion from the region commonly skipped in revertant dystrophin. This, together with exon skipping in two noncontiguous regions, favors aberrant splicing as the mechanism for the restoration of dystrophin, but is hard to reconcile with the clonal idiosyncrasy of revertant dystrophins. Revertant dystrophins retain functional domains and mediate plasmalemmal assembly of the dystrophin-associated glycoprotein complex. Physiological function of revertant fibers is demonstrated by the clonal growth of revertant clusters with age, suggesting that revertant dystrophin could be used as a guide to the construction of dystrophin expression vectors for individual gene therapy . The dystrophin gene in the mdx mouse provides a favored system for study of exon skipping associated with nonsense mutations.

Record Date Created: 20000425

31/7/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10435508 99426537 PMID: 10498249

Retroviral transfer of human MDR1 gene to hematopoietic cells: effects of drug selection and of transcript splicing on expression of encoded P-glycoprotein.

Licht T; Aran J M; Goldenberg S K; Vieira W D; Gottesman M M; Pastan I

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255, USA.

Human gene therapy (UNITED STATES) Sep 1 1999, 10 (13) p2173-85, ISSN 1043-0342 Journal Code: 9008950

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Protection of hematopoietic cells of patients undergoing anticancer chemotherapy by MDR1 gene transfer is currently being studied in clinical trials. From animal studies, it has been suggested that aberrant splicing due to cryptic donor and acceptor sites in the MDR1 cDNA could

be a major reason for failure to obtain high-level expression of P-glycoprotein in bone marrow. We investigated effects of drug selection on protein expression levels and on splicing of MDR1 transcripts in murine bone marrow cells (BMCs) in vitro. To this end, retroviruses were generated through an identical plasmid, pHaMDR1/A, introduced into different packaging cells. GP + E86- but not PA317-derived producer cells were found to express truncated in addition to full-length message. In BMCs transduced with GP + E86-derived viruses, both messages were increased after treatment with colchicine or daunomycin. Similar results were obtained with NIH 3T3 fibroblasts. However, transduced and drug-selected BMCs displayed the spliced transcript even if the respective PA317-derived producer cells contained no truncated RNA as detected in transduced NIH 3T3 fibroblasts. Short-term drug selection in BMCs transduced with either ecotropic or amphotropic retroviruses resulted in a striking increase in P-glycoprotein expression. Thus, aberrant splicing failed to abrogate P-glycoprotein expression in BMCs. We also studied a vector in which MDR1 was coexpressed with glucocerebrosidase, using an internal ribosomal entry site. Although chemoprotection was less efficient than with pHaMDR1/A, augmentation of protein expression was observed at low selecting drug concentrations. Our study shows that drug selection can partially compensate for inefficient transduction of hematopoietic cells, and may help to develop strategies by which unstable expression of transduced genes can be overcome.

Record Date Created: 19991027

31/7/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10364772 99362919 PMID: 10432284

Selective histocompatibility leukocyte antigen (HLA)-A2 loss caused by aberrant pre-mRNA splicing in 624MEL28 melanoma cells.

Wang Z; Marincola F M; Rivoltini L; Parmiani G; Ferrone S

Department of Immunology, Roswell Park Cancer Institute, Buffalo, New York 14263, USA.

Journal of experimental medicine (UNITED STATES) Jul 19 1999, 190 (2) p205-15, ISSN 0022-1007 Journal Code: 2985109R

Contract/Grant No.: CA67108; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Histocompatibility leukocyte antigen (HLA)-A2 is used as a restricting element to present several melanoma-associated antigen (MAA)-derived peptides to cytotoxic T lymphocytes (CTLs). HLA-A2 antigen is selectively lost in primary melanoma lesions and more frequently in metastases. Only scanty information is available about the molecular mechanisms underlying this abnormality, in spite of its potentially negative impact on the clinical course of the disease and on the outcome of T cell-based immunotherapy. Therefore, in this study we have shown that the selective HLA-A2 antigen loss in melanoma cells 624MEL28 is caused by a splicing defect of HLA-A2 pre-mRNA because of a base substitution at the 5' splice donor site of intron 2 of the HLA-A2 gene. As a result, HLA-A2 transcripts are spliced to two aberrant forms, one with exon 2 skipping and the other with intron 2 retention. The latter is not translated because of an early premature stop codon in the retained intron. In contrast, the transcript with exon 2 skipping is translated to a truncated HLA-A2 heavy

chain without the alpha(1) domain. Such a polypeptide is synthesized in vitro but is not detectable in cells, probably because of the low steady state level of the corresponding mRNA and the low translation efficiency. These results indicate that a single mutational event in an HLA class I gene is sufficient for loss of the corresponding allele. This may account, at least in part, for the high frequency of selective HLA class I allele loss in melanoma cells. Our conclusion emphasizes the need to implement active specific immunotherapy with a combination of peptides presented by various HLA class I alleles. This strategy may counteract the ability of melanoma cells with selective HLA class I allele loss to escape from immune recognition.

Record Date Created: 19990816

31/7/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10345897 99336194 PMID: 10407856

Specific removal of the nonsense mutation from the mdx dystrophin mRNA using antisense oligonucleotides.

Wilton S D; Lloyd F; Carville K; Fletcher S; Honeyman K; Agrawal S; Kole R

Department of Pathology, Australian Neuromuscular Research Institute, University of Western Australia, QE II Medical Centre, Nedlands, Australia. swilton@cyllene.uwa.edu.au

Neuromuscular disorders : NMD (ENGLAND) Jul 1999, 9 (5) p330-8, ISSN 0960-8966 Journal Code: 9111470

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The mdx mouse, which carries a nonsense mutation in exon 23 of the dystrophin gene, has been used as an animal model of Duchenne muscular dystrophy to evaluate cell or gene replacement therapies . Despite the mdx mutation, which should preclude the synthesis of a functional dystrophin protein, rare, naturally occurring dystrophin-positive fibres have been observed in mdx muscle tissue. These dystrophin-positive fibres are thought to have arisen from an exon-skipping mechanism, either somatic mutations or alternative splicing . Increasing the frequency of these fibres may offer another therapeutic approach to reduce the severity of Duchenne muscular dystrophy. Antisense oligonucleotides have been shown to block aberrant splicing in the human beta-globin gene. We wished to use a similar approach to re-direct normal processing of the dystrophin pre-mRNA and induce specific exon skipping. Antisense 2'-O-methyl-oligoribonucleotid es, directed to the 3' and 5' splice sites of introns 22 and 23, respectively in the mdx pre-mRNA, were used to transfect myoblast cultures. The 5' antisense oligonucleotide appeared to efficiently displace factors normally involved in the removal of intron 23 so that exon 23 was also removed during the splicing of the dystrophin pre-mRNA. Approximately 50% of the dystrophin gene mRNAs were missing this exon 6 h after transfection of primary mdx myotubes, with all transcripts showing skipping of exon 23 after 24 h. Deletion of exon 23 does not disrupt the reading frame and should allow the synthesis of a shorter but presumably functional Becker-like dystrophin. Molecular intervention at dystrophin pre-mRNA splicing has the potential to reduce the severity of a Duchenne mutation to the milder Becker phenotype.

Record Date Created: 19990825

31/7/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10323610 99311000 PMID: 10381826

Fluorescent PCR and automated fragment analysis in preimplantation genetic diagnosis for 21-hydroxylase deficiency in congenital adrenal hyperplasia.

Van de Velde H; Sermon K; De Vos A; Lissens W; Joris H; Vandervorst M; Van Steirteghem A; Liebaers I

Centre for Reproductive Medicine, University Hospital and Medical School, Dutch-speaking Brussels Free University (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium.

Molecular human reproduction (ENGLAND) Jul 1999, 5 (7) p691-6, ISSN 1360-9947 Journal Code: 9513710

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease which is most often caused by a deficiency in steroid 21-hydroxylase. The disease is characterized by a range of impaired adrenal cortisol and aldosterone synthesis combined with an increased androgen synthesis. These metabolic abnormalities lead to an inability to conserve sodium and virilization of females. The most common mutation causing the severe form of CAH is a conversion of an A or C at nucleotide (nt) 656 to a G in the second intron of the steroid 21-hydroxylase gene (CYP21) causing aberrant splicing of mRNA. A couple was referred to our centre for preimplantation genetic diagnosis (PGD) for 21-hydroxylase deficiency in CAH. A PGD was set up to detect the nt656 A/C-->G mutation using fluorescent polymerase chain reaction (PCR) and subsequent restriction enzyme digestion and fragment analysis on an automated sequencer. Using DNA or single cells from the father, the normal allele could not be amplified. Non-amplification of the normal allele has been previously described in asymptomatic carriers, therefore the PCR was further developed using heterozygous lymphoblasts from the mother. The PCR was shown to be highly efficient (96% amplification), accurate (0% contamination) and reliable (0% allelic drop-out). The couple started PGD treatment and the second PCR cycle resulted in a twin pregnancy. The genotype of the fetuses was determined in our laboratory using chorionic villus sampling material using the method described here. Both fetuses were shown to be heterozygous carriers of the mutation, and two healthy girls were born.

Record Date Created: 19990816

31/7/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09805833 98241344 PMID: 9572837

Up-regulation of luciferase gene expression with antisense oligonucleotides: implications and applications in functional assay development.

Kang S H; Cho M J; Kole R

School of Pharmacy, Department of Pharmacology, and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599, USA.

Biochemistry (UNITED STATES) May 5 1998, 37 (18) p6235-9, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM50768; GM; NIGMS; HL32352; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

HeLa Tet-Off cells were transfected transiently as well as stably with a recombinant plasmid (pLuc/705) carrying the luciferase gene interrupted by a mutated human beta-globin intron 2 (IVS2-705). The mutation in the intron causes aberrant splicing of luciferase pre-mRNA, preventing translation of luciferase. However, treatment of the cells with a 2'-O-methyl-oligoribonucleotide targeted to the aberrant splice sites induces correct splicing , restoring luciferase activity. The effects are sequence-specific, depend on the concentration of the oligonucleotide, and can be modulated by the pretreatment of the cell line, Luc/705, with tetracycline. Thus, the cell line provides, among others, a novel functional assay system superior to other procedures that are based on protein down-regulation. In particular, the system would be ideal in assessing the cellular delivery efficiency of antisense oligonucleotides.

Record Date Created: 19980608

31/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09754569 98183763 PMID: 9523162

Evaluation of soluble CD44 splice variant v5 in the diagnosis and follow-up in breast cancer patients.

Kittl E M; Ruckser R; Selleny S; Samek V; Hofmann J; Huber K; Reiner A; Ogris E; Hinterberger W; Bauer K
Institute of Laboratory Medicine Donauspital, Vienna, Austria.

Experimental and clinical immunogenetics (SWITZERLAND) 1997, 14 (4) p264-72, ISSN 0254-9670 Journal Code: 8411714
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Aberrant expression of CD44 splice variants has been detected on a variety of human tumor cells. Overexpression of specific isoforms has been shown to be associated with metastasis and poor prognosis in breast cancer. We evaluated the possible utility of soluble CD44 splice variant v5 (sCD44v5) as a circulating, tumor-associated marker in breast cancer patients. Serum levels of sCD44v5 were determined in 147 healthy volunteers, in 53 patients with nonmalignant breast disease, in 85 patients with breast cancer at presentation, in 13 patients with recurrence and in 73 patients with active metastatic disease..Statistically, the levels at presentation in stages I-IV, in benign disease, and in a female control group were not significantly different. First longitudinal studies over 1-2 years in the follow-up of 28 patients who have remained tumor-free showed considerable between-patient variation while the intrapatient levels remained within relatively narrow limits. In patients with active metastatic disease, elevated levels of sCD44v5 (> 58 ng.ml-1) were detected in 50% of the cases with marked elevation in only 26%. In these cases, sCD44v5 correlated with the extent of metastatic disease and fell during clinical response to cytoreductive therapy . In comparison with CA15-3 in the patients' follow-up serum levels of sCD44v5 proved to be much less

sensitive concerning lead time, percentage of raised serum levels at the time of recurrence and in metastatic disease. The value of sCD44v5 determinations in breast cancer patients was further limited by the poor diagnostic specificity of this marker due to elevated levels in smokers and chronic inflammatory disease.
Record Date Created: 19980514

31/7/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09172018 97075079 PMID: 8917506
Repair of thalassemic human beta-globin mRNA in mammalian cells by antisense oligonucleotides.
Sierakowska H; Sambade M J; Agrawal S; Kole R
Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill 27599, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 12 1996, 93 (23) p12840-4, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In one form of beta-thalassemia, a genetic blood disorder, a mutation in intron 2 of the beta-globin gene (IVS2-654) causes aberrant splicing of beta-globin pre-mRNA and, consequently, beta-globin deficiency. Treatment of mammalian cells stably expressing the IVS2-654 human beta-globin gene with antisense oligonucleotides targeted at the aberrant splice sites restored correct splicing in a dose-dependent fashion, generating correct human beta-globin mRNA and polypeptide. Both products persisted for up to 72 hr posttreatment. The oligonucleotides modified splicing by a true antisense mechanism without overt unspecific effects on cell growth and splicing of other pre-mRNAs. This novel approach in which antisense oligonucleotides are used to restore rather than to down-regulate the activity of the target gene is applicable to other splicing mutants and is of potential clinical interest.
Record Date Created: 19961230

31/7/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07859461 93390554 PMID: 7690899
Insertional inactivation of the tk locus in a human B lymphoblastoid cell line by a retroviral shuttle vector.
Grosovsky A J; Skandalis A; Hasegawa L; Walter B N
Evironmental Toxicology Graduate Program, University of California, Riverside 92521.
Mutation research (NETHERLANDS) Oct 1993, 289 (2) p297-308, ISSN 0027-5107 Journal Code: 0400763
Contract/Grant No.: RO1 CA55659; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Insertional mutagenesis represents an inherent risk in retrovirally

mediated gene therapy , but it may be a useful experimental strategy for identification and isolation of novel cellular loci. In this investigation we have established a model system using a heterozygous thymidine kinase (tk) marker locus in a human B lymphoblastoid cell line, and a M-MuLV based shuttle vector. The frequency of TK- mutants in cells carrying 1-2 proviruses per genome is approximately 2 x 10(-5), a 5-fold increase as compared to an uninfected control population. Southern analysis of a set of 13 retrovirus infected TK- mutants revealed a predominance of rearrangements among those mutants which had not undergone loss of heterozygosity. No consistent relationship was found to exist between the occurrence of a rearrangement and tk gene expression as detected by northern analysis. The mechanisms of retroviral shuttle vector insertional mutagenesis were characterized in more detail by focusing on a single TK- mutant, T2. The single proviral insert in T2 was found to lie within tk intron 2, in parallel orientation to the direction of tk transcription. DNA sequence analysis of tk cDNA revealed the presence of an aberrantly spliced product from which exon 4 is excluded. Aberrant splicing could sufficiently account for the low level of functional tk transcript and thus the TK- phenotype in T2, although potential contributions from other mechanisms cannot be excluded.

Record Date Created: 19931015

31/7/16 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13341169 BIOSIS NO.: 200100548318
Identification of splice variants in Amyotrophic Lateral Sclerosis using a new technology: DATAS.
AUTHOR: Ait-Ikhlef A(a); Resink A M(a); Bourdin J(a); Marcade M(a); Schweighoffer F(a)
AUTHOR ADDRESS: (a)Exonhit Therapeutics, Paris**France
JOURNAL: Society for Neuroscience Abstracts 27 (2):p1649 2001
MEDIUM: print
CONFERENCE/MEETING: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001
ISSN: 0190-5295
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of the large motoneurons of the spinal cord, brain stem and cortex. About 20% of familial ALS (FALS which represents 5 to 10% of ALS cases) are linked to mutations in the enzyme superoxide-dismutase 1 (SOD-1). The etiology of the disorder is still unknown. Studies of specific genes have shown that aberrant RNA splicing occured in neurodegenerative diseases. We developped an original technology called DATAS (Differential Analysis of Transcripts with Alternative Splicing) aimed to detect the deregulation of RNA splicing process. This technology enabled us to identify RNA splicing variants in SOD-1 transgenic mice, a model of ALS. We performed DATAS in SOD-1 mice of the age of 30, 60 and 90 days resulting in the isolation of spliced fragment of different genes involved in many cellular pathways. Among those genes, some are already described to participate to apoptosis during neurodegeneration, others

are involved in the mitochondria function which modifications are one of the first event affected in ALS. Splice variants of three genes were further characterized by conventional techniques. Results will be presented showing an innovative approach to identify new therapeutic targets.

31/7/17 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11134335 BIOSIS NO.: 199799755480
Expression of HuD (a paraneoplastic encephalomyelitis antigen) mRNA in lung cancer.
AUTHOR: Cho Jeong-Hee(a); Noguchi Masayuki
AUTHOR ADDRESS: (a)Dep. Pathol., Coll. Med., Dankook Univ., 29 Anseo-dong, Cheonan, Chungnam 330-714**South Korea
JOURNAL: Journal of Korean Medical Science 12 (4):p305-310 1997
ISSN: 1011-8934
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: HuD, one of the Hu antigens (HuD and HuC), was recognized in the sera of small cell lung cancer (SCLC) patients with antibody-associated paraneoplastic encephalomyelitis/peripheral sensory neuropathy (PEM/PSN). Three forms of HuD mRNA, 197, 156, 110 nucleotides are made by alternative splicing at 868909 residues and an additional 3'-splice site. To determine the diagnostic value of the HuD expression for small cell lung cancer, we examined 4 SCLC cell lines, 9 surgically resected SCLCs, and 12 surgically resected non-SCLCs using the reverse transcriptase-polymerase chain reaction with the HuD-specific primer pairs that spanned the putative alternative 3'- splicing site and direct DNA sequencing. None of the patients were associated with PEM/PSN. A single RNA transcript (156 nucleotides) among three forms (110, 156, 197 nucleotides) of the HuD gene was an alternatively spliced at 868-909 residues in SCLC cell lines. Expression of the HuD gene was stronger in three classic cell lines, but not in a variant cell line. Two of 9 SCLCs (22%) and 3 of 12 non-SCLCs (25%) expressed only the major RNA transcript [illegible] same fashion as the cell line. These results revealed that no aberrant alternative splicing occurred in SCLC not associated with PEM/PSN and the expression of HuD gene was not specific for a particular histologic subtype of human lung cancer.

31/7/18 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09097494 BIOSIS NO.: 199497105864
Abnormal alternative splicing of erythroid ankyrin mRNA in two kindred with hereditary spherocytosis (ankyrin-PRAGUE and ankyrin-RAKOVNIK).
AUTHOR: Jarolim P(a); Rubin H L; Brabec V; Palek J
AUTHOR ADDRESS: (a)Dep. Biomed. Res., St. Elizabeth's Med. Cent., Tufts Univ. Sch. Med., Boston, MA**USA
JOURNAL: Blood 82 (10 SUPPL. 1):p5A 1993

CONFERENCE/MEETING: Thirty-fifth Annual Meeting of the American Society of Hematology St. Louis, Missouri, USA December 3-7, 1993
ISSN: 0006-4971
RECORD TYPE: Citation
LANGUAGE: English

31/7/19 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

11608200 EMBASE No: 2002179696
Adenosine deaminase deficiency with mosaicism for a "second-site suppressor" of a splicing mutation: Decline in revertant T lymphocytes during enzyme replacement therapy
Arredondo-Vega F.X.; Santisteban I.; Richard E.; Bali P.; Koleilat M.; Loubser M.; Al-Ghonaium A.; Al-Helali M.; Hershfield M.S.
M.S. Hershfield, Box 3049, Duke University Medical Center, Durham, NC 27710 United States
AUTHOR EMAIL: msh@biochem.duke.edu
Blood (BLOOD) (United States) 01 FEB 2002, 99/3 (1005-1013)
CODEN: BLOOA ISSN: 0006-4971
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 58

Four patients from 3 Saudi Arabian families had delayed onset of immune deficiency due to homozygosity for a novel intronic mutation, g.31701T>A, in the last splice acceptor site of the adenosine deaminase (ADA) gene. Aberrant splicing mutated the last 4 ADA amino acids and added a 43-residue "tail" that rendered the protein unstable. Mutant complementary DNA (cDNA) expressed in Escherichia coli yielded 1% of the ADA activity obtained with wild-type cDNA. The oldest patient, 16 years old at diagnosis, had greater residual immune function and less elevated erythrocyte deoxyadenosine nucleotides than his 4-year-old affected sister. His T cells and Epstein-Barr virus (EBV) B cell line had 75% of normal ADA activity and ADA protein of normal size. DNA from these cells and his whole blood possessed 2 mutant ADA alleles. Both carried g.31701T>A, but one had [illegible] suppressed aberrant splicing and excised an unusual purine-rich tract from the wild-type intron 11/exon 12 junction. During ADA replacement therapy , ADA activity in T cells and abundance of the "second-site" revertant allele decreased markedly. This finding raises an important issue relevant to stem cell gene therapy . (c) 2002 by The American Society of Hematology.

31/7/20 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

07298615 EMBASE No: 1998196062
A case of myelodysplastic syndrome with an intronic point mutation of the p53 tumour suppressor gene at the splice donor site
Kikukawa M.; Aoki N.; Mori M.
Dr. M. Mori, Department of Haematology, Tokyo Metropolitan Geriatric Hosp., 35-2 Sakaechou, Itabashiku, Tokyo 173 Japan

British Journal of Haematology (BR. J. HAEMATOL.) (United Kingdom)
1998, 100/3 (564-566)
CODEN: BJHEA ISSN: 0007-1048
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 12

We analysed genomic DNA and mRNA of the p53 gene in a case of myelodysplastic syndrome (MDS) with monosomy of chromosome 17. DNA analysis revealed a mutation at the splice donor site. (GT to GC) of intron 5. mRNA analysis revealed the presence of abnormal splicing with 46 nucleotide deletion in exon 5, producing a downstream frame shift and a predicted truncated protein which lacked normal function. The p53 gene mutation at the splice donor site contributes to the inactivation of the p53 gene function and may play an important role in the pathogenesis, progression and therapeutic responsiveness of MDS.
?logoff hold

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show files
File 155:MEDLINE(R) 1966-2002/Aug W3
File   5:Biosis Previews(R) 1969-2002/Aug W2
         (c) 2002 BIOSIS
File 315:ChemEng & Biotec Abs 1970-2002/Jul
         (c) 2002 DECHEMA
File  73:EMBASE 1974-2002/Aug W3
         (c) 2002 Elsevier Science B.V.
?ds

Set     Items   Description
S1    8062370   TREAT? OR THERAP?
S2       3456   (ABNORMAL OR ABERRANT OR DEFECT?)(5N)SPLIC?
S3     775225   EXPRESS? (5N) GENE?
S4      27183   ALTERNATIVE(3N)SPLIC?
S5      57577   CYSTIC()FIBROSIS
S6     939501   MUTATION? ? OR MUTANT? ?
S7       2254   SR(2N)PROTEIN? ?
S8        229   HETEROGENEOUS(3N)NUCLEAR(3N)RIBONUCLEOPROTEIN(3N)A1
S9         10   VIRAL(3N)FACTOR? ?(3N)E4?
S10       534   HNRNP(2N)A1
S11      1355   SR()PROTEIN? ?
S12       394   S11 AND S4
S13       116   S12 AND S3
S14         9   S13 AND S1
S15         5   S13 AND (DISORDER? ? OR DISEAS? OR SYNDROME OR ILLNESS? ?)
S16       116   S2 AND S3 AND S4
S17       229   S13 OR S16
S18         0   1 S2 AND S5
S19       121   S10 AND S4
S20         3   S19 AND S5
S21        34   S19 AND S3
S22        36   S2 AND CFTR
S23        91   S9 OR S14 OR S15 OR S18 OR S20 OR S21 OR S22
S24        48   RD S23 (unique items)
S25       220   AU=KEREM B? OR AU=KEREM, B?
S26         3   S25 AND S2
S27        50   S24 OR S26
?t 28/7/all

 28/7/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12845473   21668184   PMID: 11810271
  Qualitative and quantitative analysis of mRNA associated with four
putative splicing mutations (621+3A-->G, 2751+2T-->A, 296+1G-->C,
1717-9T-->C-D565G) and one nonsense mutation (E822X) in the CFTR gene.
  Tzetis M; Efthymiadou A; Doudounakis S; Kanavakis E
  Department of Medical Genetics, Athens University, Aghia Sophia
Children's Hospital, Thivon & Livadias, Athens, 11527, Greece.
  Human genetics (Germany) Dec 2001, 109 (6) p592-601, ISSN 0340-6717
Journal Code: 7613873
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
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The effects of four splicing mutations and one nonsense mutation on cystic fibrosis transmembrane conductance regulator (CFTR) gene expression were investigated by reverse transcription-polymerase chain reaction analysis of mRNA extracted from nasal epithelial cells harvested from patients harbouring the mutations. We studied four subjects with 621+3A-->G, two with 2751+2T-->A, one with 296+1G-->C, two with 1717-9T-->C-D565G and seven with E822X and compared the results with CFTR mRNA from normal subjects. Our results showed that mutations 621+3A-->G, 2751+2T-->A, and 296+1G-->C, which disrupt the 5' splice donor sites of introns 4, 14a, and 2, respectively, and 1717-9T-->C-D565G, which possibly disrupts the exonic splicing enhancer sequences of exon 12 (owing to the missense mutation in cis), lead to the production of aberrantly spliced mRNA in nasal epithelial cells. Three of the splicing mutations (621+3A-->G, 2751+2T-->A, and 296+1G-->C) result in severe deficiency of normal CFTR mRNA and severe phenotype in the patients. This information is especially useful for mutation 621+3A-->G, which is found in other populations as well, and was initially reported as a polymorphism. The complex allele 1717-9T-->C-D565G results in aberrant splicing of CFTR mRNA with production of transcripts lacking exon 12 (major product), with minor amounts of transcripts revealing joint exon 11 and 12 skipping. Nonsense mutation E822X results in a severe reduction in mRNA levels to about 6% of wild type. Patients with the mutation have a severe clinical phenotype, with both the pancreatic and the pulmonary function affected.

Record Date Created: 20020125

28/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12575915 21481770 PMID: 11598017

The hnRNP A1 protein regulates HIV-1 tat splicing via a novel intron silencer element.

Tange T O; Damgaard C K; Guth S; Valcarcel J; Kjems J

Department of Molecular and Structural Biology, University of Aarhus, C.F. Mollers Alle, Building 130, DK-8000 Arhus C, Denmark.

EMBO journal (England) Oct 15 2001, 20 (20) p5748-58, ISSN 0261-4189 Journal Code: 8208664

Document type: Journal Article

Main Citation Owner: NLM

Record type: Completed

The generation of >30 different HIV-1 mRNAs is achieved by alternative splicing of one primary transcript. The removal of the second tat intron is regulated by a combination of a suboptimal 3' splice site and cis-acting splicing enhancers and silencers. Here we show that hnRNP A1 inhibits splicing of this intron via a novel heterogeneous nuclear ribonucleoprotein (hnRNP) A1 -responsive intron splicing silencer (ISS) that can function independently of the previously characterized exon splicing silencer (ESS3). Surprisingly, depletion of hnRNP A1 from the nuclear extract (NE) enables splicing to proceed in NE that contains 100-fold reduced concentrations of U2AF and normal levels of SR proteins, conditions that do not support processing of other efficiently spliced pre-mRNAs. Reconstituting the extract with recombinant hnRNP A1 protein restores splicing inhibition at a step subsequent to U2AF binding, mainly at the time of U2 snRNP association. hnRNP A1 interacts specifically with the ISS sequence, which overlaps with one of three alternative branch point sequences, pointing to a model where the entry of U2 snRNP is physically

blocked by hnRNP A1 binding.
Record Date Created: 20011012

28/7/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11361602 21443304 PMID: 11559564
Specific inhibition of serine- and arginine-rich splicing factors phosphorylation, spliceosome assembly, and splicing by the antitumor drug NB-506.
Pilch B; Allemand E; Facompre M; Bailly C; Riou J F; Soret J; Tazi J
Institut de Genetique Moleculaire, UMR 5535, Centre National de la Recherche Scientifique, IFR 24, Universite de Montpellier II, 34293 Montpellier Cedex, France.
Cancer research (United States) Sep 15 2001, 61 (18) p6876-84, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Specific phosphorylation of serine- and arginine-rich pre-mRNA splicing factors (SR proteins) is one of the key determinants regulating splicing events. Several kinases involved in SR protein phosphorylation have been identified and characterized, among which human DNA topoisomerase I is known to have DNA-relaxing activity. In this study, we have investigated the mechanism of splicing inhibition by a glycosylated indolocarbazole derivative (NB-506), a potent inhibitor of both kinase and relaxing activities of topoisomerase I. NB-506 completely inhibits the capacity of topoisomerase I to phosphorylate, in vitro, the human splicing factor 2/ alternative splicing factor (SF2/ASF). This inhibition is specific, because NB-506 does not demonstrate activity against other kinases known to phosphorylate SF2/ASF such as SR protein kinase 1 and cdc2 kinase. Importantly, HeLa nuclear extracts competent in splicing but not splicing-deficient cytoplasmic S100 extracts treated with the drug fail to phosphorylate SF2/ASF and to support splicing of pre-mRNA substrates containing SF2/ASF-target sequences. Native gel analysis of splicing complexes revealed that the drug affects the formation of the [redacted] place. In the presence of the drug, neither pre-spliceosome nor spliceosome is formed, demonstrating that splicing inhibition occurs at early steps of spliceosome assembly. Splicing inhibition can be relieved by adding phosphorylated SF2/ASF, showing that extracts treated with NB-506 lack a phosphorylating activity required for splicing. Moreover, NB-506 has a cytotoxic effect on murine P388 leukemia cells but not on P388CPT5 camptothecin-resistant cells that carry two point mutations in conserved regions of topoisomerase I gene (Gly361Val and Asp709Tyr). After drug treatment , P388 cells accumulated hypophosphorylated forms of SR proteins and polyadenylated RNA in the nucleus. In contrast, neither SR protein phosphorylation nor polyadenylated mRNA distribution was affected in P388 CPT5- treated cells. Consistently, NB506 treatment altered the mRNA levels and/or splicing pattern of several tested genes (Bcl-X, CD 44, SC35, and Sty) in P388 cells but not in P388 CPT5 cells. The study shows for the first time that indolocarbazole drugs targeting topoisomerase I can affect gene expression by modulating pre-mRNA splicing through inhibition of SR proteins phosphorylation.
Record Date Created: 20010917

28/7/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11229098 21264529 PMID: 11278983
Dimethyl sulfoxide affects the selection of splice sites.
Bolduc L; Labrecque B; Cordeau M; Blanchette M; Chabot B
Departement de Microbiologie et d'Infectiologie, Faculte de Medecine, Universite de Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4.
Journal of biological chemistry (United States) May 18 2001, 276 (20) p17597-602, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Depending on the cell lines and cell types, dimethyl sulfoxide (Me2SO) can induce or block cell differentiation and apoptosis. Although Me2SO treatment alters many levels of gene expression , the molecular processes that are directly affected by Me2SO have not been clearly identified. Here, we report that Me2SO affects splice site selection on model pre-mRNAs incubated in a nuclear extract prepared from HeLa cells. A shift toward the proximal pair of splice sites was observed on pre-mRNAs carrying competing 5'-splice sites or competing 3'-splice sites. Because the activity of recombinant hnRNP A1 protein was similar when added to extracts containing or lacking Me2SO, the activity of endogenous A1 proteins is probably not affected by Me2SO. Notably, in a manner reminiscent of SR proteins , Me2SO activated splicing in a HeLa S100 extract. Moreover, the activity of recombinant SR proteins in splice site selection in vitro was improved by Me2SO. Polar solvents like DMF and formamide similarly modulated splice site selection in vitro but formamide did not activate a HeLa S100 extract. We propose that Me2SO improves ionic interactions between splicing factors that contain RS-domains. The direct impact of Me2SO on alternative splicing may explain, at least in part, the different and sometimes opposite effects of Me2SO on cell differentiation and apoptosis.
Record Date Created: 20010523

28/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11179576 21192921 PMID: 11295081
Transformation-associated changes in gene expression of alternative splicing regulatory factors in mouse fibroblast cells.
Maeda T; Furukawa S
Department of Radiology and Radiation Oncology, Osaka University Graduate School of Dentistry, Osaka, Japan. tmaeda@radiol.dent.osaka-u.ac.jp
Oncology reports (Greece) May-Jun 2001, 8 (3) p563-6, ISSN 1021-335X Journal Code: 9422756
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Although the alternative splicing of various genes is a common phenomenon in tumorigenesis, little is known about the mechanism behind it. Recently, we found altered expression of splicing regulatory factors during

two-step chemical transformation in vitro. However, it remains unknown whether such altered expression of splicing factors commonly occur during other modes of transformation. We have further investigated the expression of five splicing regulatory factors, heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and A2, alternative splicing factor/ splicing factor 2 (ASF/SF2), spliceosome component of 35-kDa protein (SC35) and 65-kDa subunit of U2 snRNP auxiliary factor (U2AF65), using two series of clonally-derived mouse fibroblast cell lines, that were SV40 large T transfectants (SV-T2 and NIH3T3/13C7) or c-erbB2 transfectants (A4). U2AF65 expression was increased (2.1- and 2.7-fold) in NIH3T3/13C7 and A4 compared with the normal parental cells, respectively, and SC35 expression was increased 1.8- to 2.3-fold in all transformed cells. These results suggest that altered expression of some splicing regulatory factors may commonly occur during various modes of cellular transformation.
Record Date Created: 20010411

28/7/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11171905 21181834 PMID: 11285240
Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping.
Buratti E; Dork T; Zuccato E; Pagani F; Romano M; Baralle F E
International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34012 Trieste, Italy.
EMBO journal (England) Apr 2 2001, 20 (7) p1774-84, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Alternative splicing of human cystic fibrosis transmembrane conductance regulator (CFTR) exon 9 is regulated by a combination of cis-acting elements distributed through the exon and both flanking introns (IVS8 and IVS9). Several studies have identified in the IVS8 intron 3' splice site a regulatory element that is composed of a polymorphic (TG)m(T)n repeated sequence. At present, no cellular factors have been identified that recognize this element. We have identified TDP-43, a nuclear protein not previously described to bind RNA, as the factor binding specifically to the (TG)m sequence. Transient TDP-43 overexpression in Hep3B cells results in an increase in exon 9 skipping. This effect is more pronounced with concomitant overexpression of SR proteins. Antisense inhibition of endogenous TDP-43 expression results in increased inclusion of exon 9, providing a new therapeutic target to correct aberrant splicing of exon 9 in CF patients. The clinical and biological relevance of this finding in vivo is demonstrated by our characterization of a CF patient carrying a TG10T9(DeltaF508)/TG13T3(wt) genotype leading to a disease-causing high proportion of exon 9 skipping.
Record Date Created: 20010404

28/7/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10958996 20524252 PMID: 11069835
Lung disease associated with the IVS8 5T allele of the CFTR gene.

Noone P G; Pue C A; Zhou Z; Friedman K J; Wakeling E L; Ganeshananthan M; Simon R H; Silverman L M; Knowles M R

Cystic Fibrosis/Pulmonary Research and Treatment Center, Departments of Medicine, and Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7248, USA.

American journal of respiratory and critical care medicine (UNITED STATES) Nov 2000, 162 (5) p1919-24, ISSN 1073-449X Journal Code: 9421642

Contract/Grant No.: 34332; PHS; RR00046; RR; NCRR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. The 5T allele in intron 8 (IVS8) causes abnormal splicing in the CFTR gene, and is associated with lung disease when it occurs in cis with a missense mutation in the CFTR gene, R117H. However, the 5T variant alone has not been reported to cause lung disease. We describe two adult female patients with CF-like lung disease associated with the 5T allele. One patient's genotype is 5T-TG12-M470V/5T-TG12-M470V, and the other is DeltaF508/5T-TG12-M470V; full sequencing of the CFTR gene revealed no other mutation on the same allele as the 5T variant. The levels of full-length CFTR mRNA in respiratory epithelia were very low in these patients (11 and 6%, respectively, of total CFTR mRNA expression). Both patients had defective CFTR -mediated chloride conductance in the sweat ductal and/or acinar epithelia (sweat chloride, mmol/L, mean +/- SEM: 40.0 +/- 5.0 [n = 8 samples] and 80. 0 +/- 3.5 [n = 6 samples]) and airway epithelia (mV, mean +/- SEM CFTR -mediated Cl(-) conductance of 1.2 +/- 2.2 [n = 5 studies] and -6.75 +/- 8.1 [n = 4 studies]). These data suggest that the 5T polythymidine tract sequence on specific haplotype backgrounds (TG12 and M470V) may cause a low level of full-length functional CFTR protein and CF-like lung disease.

Record Date Created: 20001204

28/7/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10873000 20440195 PMID: 10982852

[illegible] alternative splicing : an intron element represses use of the common 3' splice site.

Simard M J; Chabot B

Departement de Microbiologie et d'Infectiologie, Faculte de Medecine, Universite de Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4.

Molecular and cellular biology (UNITED STATES) Oct 2000, 20 (19) p7353-62, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Alternative splicing of exon 7B in the hnRNP A1 pre-mRNA produces mRNAs encoding two proteins: hnRNP A1 and the less abundant A1B. We have reported the identification of several intron elements that contribute to exon 7B skipping. In this study, we report the activity of a novel element, conserved element 9 (CE9), located in the intron downstream of exon 7B. We show that multiple copies of CE9 inhibit exon 7B-exon 8 splicing in vitro. When CE9 is inserted between two competing 3' splice sites, a single copy of CE9 decreases splicing to the distal 3' splice

site. Our in vivo results also support the conclusion that CE9 is a splicing modulator. First, inserting multiple copies of CE9 into an A1 minigene compromises the production of fully spliced products. Second, one copy of CE9 stimulates the inclusion of a short internal exon in a derivative of the human beta-globin gene. In this case, in vitro splicing assays suggest that CE9 decreases splicing of intron 1, an event that improves splicing of intron 2 and decreases skipping of the short internal exon. The ability of CE9 to act on heterologous substrates, combined with the results of a competition assay, suggest that the activity of CE9 is mediated by a trans-acting factor. Our results indicate that CE9 represses the use of the common 3' splice site in the hnRNP A1 alternative splicing unit.

Record Date Created: 20001019

28/7/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10826355 20377488 PMID: 10915765

Cellular and viral splicing factors can modify the splicing pattern of CFTR transcripts carrying splicing mutations.

Nissim-Rafinia M; Chiba-Falek O; Sharon G; Boss A; Kerem B

Department of Genetics, Life Sciences Institute, The Hebrew University, Jerusalem 91904, Israel.

Human molecular genetics (ENGLAND) Jul 22 2000, 9 (12) p1771-8, ISSN 0964-6906 Journal Code: 9208958

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Variable levels of aberrantly spliced cystic fibrosis transmembrane conductance regulator (CFTR) transcripts were suggested to correlate with variable cystic fibrosis (CF) severity. We studied the effect of the cellular splicing factors, hnRNP A1 and ASF/SF2, and their adenoviral analogues, E4-ORF6 and E4-ORF3, that promote exon skipping and/or exon inclusion, on the splicing pattern of the CFTR mutation 3849+10kb C-->T and the 5T allele. These mutations can lead to cryptic exon inclusion and exon skipping, respectively. Overexpression of the cellular factors promoted exon skipping of pre-mRNA transcribed from minigenes carrying the mutation (p5T or p3849M). This led to a substantial decrease in the level of correctly spliced mRNA transcribed from p5T and generated correctly spliced mRNA transcribed from p3849M that was not found without overexpression of the factors . The viral factor , E4 -ORF3, promoted exon inclusion and led to a substantial increase of the correctly spliced mRNA transcribed from the p5T. The factor, E4-ORF6, activated exon skipping and generated correctly spliced mRNA transcribed from p3849M. Thus, overexpression of alternative splicing factors can modulate the splicing pattern of CFTR alleles carrying splicing mutations. These results are important for understanding the mechanism underlying phenotypic variability in CF and other genetic diseases.

Record Date Created: 20001027

28/7/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10744710 20300163 PMID: 10843177

Spatio-temporal expression of the trans-acting splicing factors SF2/ASF and heterogeneous ribonuclear proteins A1/A1B in the myometrium of the pregnant human uterus: a molecular mechanism for regulating regional protein isoform expression in vivo.

Pollard A J; Sparey C; Robson S C; Krainer A R; Europe-Finner G N
Department of Obstetrics and Gynecology, University of Newcastle upon Tyne, Royal Victoria Infirmary, United Kingdom. a.j.pollard@ncl.ac.uk

Journal of clinical endocrinology and metabolism (UNITED STATES) May 2000, 85 (5) p1928-36, ISSN 0021-972X Journal Code: 0375362
Contract/Grant No.: CA-13106; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Many of the human myometrial proteins associated with uterine quiescence and the switch to coordinated contractions at the onset of labor exist as alternatively spliced isoforms. There is now extensive evidence to indicate that the nuclear concentrations of the trans-acting splicing regulators SF2/ASF and hnRNP A1 /A1B are fundamental in regulating the expression of specific protein isoforms derived from alternative splicing of single precursor messenger ribonucleic acid transcripts. The question thus arose as to whether these factors were also involved in regulating the expression of specific myometrial protein species within different uterine regions during human gestation and parturition. SF2/ASF and hnRNP A1 /A1B expression was therefore determined in paired upper (corpus) and lower segment myometrial samples taken from individual women at term/during spontaneous labor and compared with nonpregnant control samples using specific monoclonal antibodies. We report that SF2/ASF levels were substantially increased in the lower uterine region, and this was associated with a parallel decrease in levels of hnRNP A1 /A1B during gestation. Conversely, the opposite pattern was observed within the upper uterine region during pregnancy, where hnRNP A1 /A1B was significantly up-regulated and SF2/ASF levels were much less than those found in the lower uterine segment. The differential expression of hnRNP A1 /A1B and SF2/ASF in the upper and lower uterine segments may have a primary role in defining the formation of specific myometrial protein species associated with the known contractile and relaxatory properties of these regions before and during parturition.

28/7/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10690729 20231883 PMID: 10769024

The MKK(3/6)-p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation.

van der Houven van Oordt W; Diaz-Meco M T; Lozano J; Krainer A R; Moscat J; Caceres J F
MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, Scotland, United Kingdom.

Journal of cell biology (UNITED STATES) Apr 17 2000, 149 (2) p307-16, ISSN 0021-9525 Journal Code: 0375356
Contract/Grant No.: CA13106; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Individual members of the serine-arginine (SR) and heterogeneous nuclear ribonucleoprotein (hnRNP) A/B families of proteins have antagonistic effects in regulating alternative splicing . Although hnRNP A1 accumulates predominantly in the nucleus, it shuttles continuously between the nucleus and the cytoplasm. Some but not all SR proteins also undergo nucleo-cytoplasmic shuttling, which is affected by phosphorylation of their serine/arginine (RS)-rich domain. The signaling mechanisms that control the subcellular localization of these proteins are unknown. We show that exposure of NIH-3T3 and SV-40 transformed green monkey kidney (COS) cells to stress stimuli such as osmotic shock or UVC irradiation, but not to mitogenic activators such as PDGF or EGF, results in a marked cytoplasmic accumulation of hnRNP A1 , concomitant with an increase in its phosphorylation. These effects are mediated by the MKK(3/6)-p38 pathway, and moreover, p38 activation is necessary and sufficient for the induction of hnRNP A1 cytoplasmic accumulation. The stress-induced increase in the cytoplasmic levels of hnRNP A/B proteins and the concomitant decrease in their nuclear abundance are paralleled by changes in the alternative splicing pattern of an adenovirus E1A pre-mRNA splicing reporter. These results suggest the intriguing possibility that signaling mechanisms regulate pre-mRNA splicing in vivo by influencing the subcellular distribution of splicing factors.
Record Date Created: 20000515

28/7/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10545311 20062828 PMID: 10593905
Correction of aberrant splicing of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by antisense oligonucleotides.
Friedman K J; Kole J; Cohn J A; Knowles M R; Silverman L M; Kole R
Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina 27599, USA.
Journal of biological chemistry (UNITED STATES) Dec 17 1999, 274 (51) p36193-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Record type: Completed
The CFTR splicing mutation 3849 + 10 kb C --> T creates a novel donor site 10 kilobases (kb) into intron 19 of the gene and is one of the more common splicing mutations that causes cystic fibrosis (CF). It has an elevated prevalence among patients with atypically mild disease and normal sweat electrolytes and is especially prominent in Ashkenazi Jews. This class of splicing mutations, reported in several genes, involves novel splice sites activated deep within introns while leaving wild-type splice elements intact. CFTR cDNA constructs that modeled the 3849 + 10 kb C --> T mutation were expressed in 3T3 mouse fibroblasts and in CFT1 human tracheal and C127 mouse mammary epithelial cells. In all three cell types, aberrant splicing of CFTR pre-mRNA was comparable to that reported in vivo in CF patients. Treatment of the cells with 2'-O-methyl phosphorothioate oligoribonucleotides antisense toward the aberrant donor and acceptor splice sites or to the retained exon-like sequence, disfavored aberrant splicing and enhanced normal processing of CFTR pre-mRNA. This antisense-mediated correction of splicing was dose- and sequence-dependent and was accompanied by increased production of CFTR

protein that was appropriately glycosylated. Antisense-mediated correction of splicing in a mutation-specific context represents a potential gene therapy modality with applicability to many inherited disorders.
Record Date Created: 20000127

28/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10307862 99308586 PMID: 10380879
Stage-specific changes in SR splicing factors and alternative splicing in mammary tumorigenesis.
Stickeler E; Kittrell F; Medina D; Berget S M
Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.
Oncogene (ENGLAND) Jun 17 1999, 18 (24) p3574-82, ISSN 0950-9232 Journal Code: 8711562
Contract/Grant No.: CA 47112; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Using a mouse model of mammary gland development and tumorigenesis we examined changes in both alternative splicing and splicing factors in multiple stages of mammary cancer. The emphasis was on the SR family of splicing factors known to influence alternative splicing in a wide variety of genes, and on alternative splicing of the pre-mRNA encoding CD44, for which alternative splicing has been implicated as important in a number of human cancers, including breast cancer. We observed step-wise increases in expression of individual SR proteins and alternative splicing of CD44 mRNA during mammary gland tumorigenesis. Individual preneoplasias differed as to their expression patterns for SR proteins , often expressing only a sub-set of the family. In contrast, tumors demonstrated a complex pattern of SR expression. Little difference was observed between neoplasias and their metastases. Alternative splicing of CD44 also changed through the disease paradigm such that tumors produced RNA containing a mixture of variable exons, whereas preneoplasias exhibited a more restricted exon inclusion pattern. In [illegible] changed little in either concentration or splicing pattern in the same cells. These data suggest alterations in relative concentrations of specific splicing factors during early preneoplasia that become more pronounced during tumor formation. Given the ability of SR proteins to affect alternative processing decisions, our results suggest that a number of pre-mRNAs may undergo changes in alternative splicing during the early and intermediate stages of mammary cancer.
Record Date Created: 19990706

28/7/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10174183 99145120 PMID: 10022522
Dual posttranscriptional targets of retinoic acid-induced gene expression .
Manji S S; Pearson R B; Pardee M; Paspaliaris V; d'Apice A; Martin T J; Ng K W

Department of Medicine, University of Melbourne, St. Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia.

Journal of cellular biochemistry (UNITED STATES) Mar 1 1999, 72 (3) p411-22, ISSN 0730-2312 Journal Code: 8205768

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Retinoic acid-induced differentiation of the pre-osteoblastic cell line, UMR 201, is associated with a marked increase in the proficiency of posttranscriptional nuclear processing of alkaline phosphatase mRNA. In this study we attempted to correlate the posttranscriptional actions of retinoic acid with changes in phosphorylation, or abundance of spliceosome components, or both. Treatment with retinoic acid for periods of < or = 4 h resulted in dephosphorylation of nuclear U1 70K protein without affecting its abundance. Peptide mapping showed that U1 70K dephosphorylation was related to the disappearance of one specific phosphopeptide out of four major U1 70K phosphopeptides. A twofold decrease in mRNA expression of an isoform of alternative splicing factor that inhibits splicing was also observed over the same period. Tumor necrosis factor-alpha, which enhances the posttranscriptional action of retinoic acid, reduced U1 70K mRNA expression, while an inhibition of retinoic acid action by transforming growth factor-beta was associated with a marked increase in U1 70K mRNA levels. Our results draw attention to the complex interactions between short- and long-term alterations in the abundance and functional status of U1 70K, as well as SR proteins by growth and/or differentiation factors in the regulation of spliceosome formation and function.

Record Date Created: 19990429

28/7/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10101633 99102639 PMID: 9847386

Polypyrimidine tract-binding protein binds to the leader RNA of mouse hepatitis virus and serves as a regulator of viral transcription.

Li H P; Huang P; Park S; Lai M M

Department of Molecular Microbiology and Immunology, University of Southern California School of Medicine, Los Angeles, California 90033-1054 USA.

Journal of virology (UNITED STATES) Jan 1999, 73 (1) p772-7, ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A cellular protein, previously described as p55, binds specifically to the plus strand of the mouse hepatitis virus (MHV) leader RNA. We have purified this protein and determined by partial peptide sequencing that it is polypyrimidine tract-binding protein (PTB) (also known as heterogeneous nuclear ribonucleoprotein [hnRNP] I), a nuclear protein which shuttles between the nucleus and cytoplasm. PTB plays a role in the regulation of alternative splicing of pre-mRNAs in normal cells and translation of several viruses. By UV cross-linking and immunoprecipitation studies using cellular extracts and a recombinant PTB, we have established that PTB binds to the MHV plus-strand leader RNA specifically. Deletion analyses of the leader RNA mapped the PTB-binding site to the UCUAA pentanucleotide

repeats. Using a defective-interfering RNA reporter system, we have further shown that the PTB-binding site in the leader RNA is critical for MHV RNA synthesis. This and our previous study (H.-P. Li, X. Zhang, R. Duncan, L. Comai, and M. M. C. Lai, Proc. Natl. Acad. Sci. USA 94:9544-9549, 1997) combined thus show that two cellular hnRNPs, PTB and hnRNP A1 , bind to the transcription-regulatory sequences of MHV RNA and may participate in its transcription.

Record Date Created: 19990128

28/7/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09855646 98292203 PMID: 9630249

Regulated tissue-specific expression of antagonistic pre-mRNA splicing factors.

Hanamura A; Caceres J F; Mayeda A; Franza B R; Krainer A R
Cold Spring Harbor Laboratory, New York 11724-2208, USA.

RNA (New York, N.Y.) (UNITED STATES) Apr 1998, 4 (4) p430-44, ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: CA13106; CA; NCI; GM42699; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The SR proteins are essential metazoan pre-mRNA splicing factors that can also influence the selection of alternative 5' splice sites in a concentration-dependent manner. Their activity in alternative splicing in vitro is antagonized by members of the hnRNP A/B family of proteins. The opposite effects of members of these two families of antagonistic splicing factors in vitro and upon overexpression in vivo suggest that changes in their relative levels may be a natural mechanism for the regulation of alternative splicing in vivo. One prediction of this model is that the ratios of these antagonists should vary in different cell types and in other situations in which cellular or viral transcripts are differentially spliced. We raised monoclonal antibodies specific for SF2/ASF and used them to measure the abundance of SF2/ASF protein and its isoforms, its phosphorylation state in vivo and during splicing in vitro, and its association with the spliceosome. SF2/ASF exists predominantly or exclusively in a highly phosphorylated state in vivo in all cell types examined, and unphosphorylated protein was not detectable. Unphosphorylated recombinant SF2/ASF becomes rapidly phosphorylated under splicing conditions in HeLa cell extracts and associates stably with one or more exons of beta-globin pre-mRNA. This interaction appears to persist through the splicing reaction and SF2/ASF remains bound to spliced mRNA. We compared the distribution of SF2/ASF to that of its antagonist, hnRNP A1 , in different rat tissues and in immortal and transformed cell lines. We found that the protein levels of these antagonistic splicing factors vary naturally over a very wide range, supporting the notion that changes in the ratio of these proteins can affect alternative splicing of a variety of pre-mRNAs in vivo.

Record Date Created: 19980630

28/7/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09512730 97420717 PMID: 9275159
Heterogeneous nuclear ribonucleoprotein A1 binds to the transcription-regulatory region of mouse hepatitis virus RNA.
Li H P; Zhang X; Duncan R; Comai L; Lai M M
Department of Molecular Microbiology and Immunology, University of Southern California Schools of Medicine, Los Angeles, CA 90033, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Sep 2 1997, 94 (18) p9544-9, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: 19244; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A cellular protein, previously described as p35/38, binds to the complementary (-)-strand of the leader RNA and intergenic (IG) sequence of mouse hepatitis virus (MHV) RNA. The extent of the binding of this protein to IG sites correlates with the efficiency of the subgenomic mRNA transcription from that IG site, suggesting that it is a requisite transcription factor. We have purified this protein and determined by partial peptide sequencing that it is heterogeneous nuclear ribonucleoprotein (hnRNP) A1 , an abundant, primarily nuclear protein. hnRNP A1 shuttles between the nucleus and cytoplasm and plays a role in the regulation of alternative RNA splicing . The MHV(-)-strand leader and IG sequences conform to the consensus binding motifs of hnRNP A1 . Recombinant hnRNP A1 bound to these two RNA regions in vitro in a sequence-specific manner. During MHV infection, hnRNP A1 relocalizes from the nucleus to the cytoplasm, where viral replication occurs. These data suggest that hnRNP A1 is a cellular factor that regulates the RNA-dependent RNA transcription of the virus.
Record Date Created: 19971001

28/7/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09348499 97240387 PMID: 9085847
A highly stable duplex structure sequesters the 5' splice site region of hnRNP A1 alternative exon 7B.
Blanchette M; Chabot B
Department de Microbiologie et Infectiologie, Faculte de Medicine, Universite de Sherbrooke, Quebec, Canada.
RNA (New York, N.Y.) (UNITED STATES) Apr 1997, 3 (4) p405-19, ISSN 1355-8382 Journal Code: 9509184
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Exon 7B in the hnRNP A1 pre-mRNA is alternatively spliced to yield A1 and A1(B), two proteins that differ in their ability to modulate 5' splice site selection. Sequencing the murine intron downstream of exon 7B revealed the existence of several regions of similarity to the corresponding human intron. In vitro splicing assays indicate that an 84-nt region (CE6IO) decreases splicing to the proximal 5' splice site in a pre-mRNA carrying the 5' splice sites of exon 7 and 7B. In vivo, the CE6IO element promotes exon 7B skipping in pre-mRNAs expressed from a mini- gene containing the hnRNP A1 alternative splicing unit. Using oligonucleotide-targeted

RNase H cleavage assays, we provide support for the existence of highly stable base pairing interactions between CE6IO and the 5' splice site region of exon 7B. Duplex formation occurs in naked pre-mRNA, resists incubation in splicing extracts, and is associated with a reduction in the assembly of U1 snRNP-dependent complexes to the 5' splice site of exon 7B. Our results demonstrate that pre-mRNA secondary structure plays an important role in promoting exon 7B skipping in the A1 pre-mRNA.
Record Date Created: 19970425

28/7/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08882082 96240630 PMID: 8659131
A conserved region of unknown function participates in the recognition of E2F family members by the adenovirus E4 ORF 6/7 protein.
Jost C A; Ginsberg D; Kaelin W G
Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.
Virology (UNITED STATES) Jun 1 1996, 220 (1) p78-90, ISSN 0042-6822 Journal Code: 0110674
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
E2F DNA-binding activity in vivo is due to heterodimer formation between members of the E2F and DP transcription factor families. The ability of these heterodimers to serve as transcriptional regulators is modulated by complex formation with additional proteins such as the products of the retinoblastoma gene and the adenovirus E4 ORF 6/7. Each of the E2F family members cloned to date contains a highly conserved region of unknown function, termed the marked box, which lies between their DNA binding and transactivation domains. Mutational analysis showed that the marked box contributed to the recognition of E2F family members by the E4 ORF 6/7 protein in vitro and in vivo.
Record Date Created: 19960801

28/7/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08871417 96221982 PMID: 8637006
Transactivation of adenovirus E2-early promoter by E1A and E4 6/7 in the context of viral chromosome.
Swaminathan S; Thimmapaya B
Robert H. Lurie Cancer Center and Microbiology-Immunology Department Northwestern University Medical School, Chicago, Illinois 60611, USA.
Journal of molecular biology (ENGLAND) May 24 1996, 258 (5) p736-46, ISSN 0022-2836 Journal Code: 2985088R
Contract/Grant No.: AL18029; PHS; AL20156; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Transcription from adenovirus E2-early promoter is controlled by a unique array of four cis-acting elements which include an atypical TBP site, two E2F sites present in an inverted orientation relative to each other, and an ATF site. In virus-infected cells, this promoter is transactivated by E1A

and the E4 6/7 proteins. In addition, it is also stimulated by the DNA-binding protein (DBP) in transient transfection assays. Here we describe a genetic analysis of the E2 transcriptional regulation in the context of the viral chromosome. By using genetically engineered mutant adenoviruses we have determined the interrelationship between the different cis-acting elements of the E2-early promoter during basal transcription, the extent to which E1A and E4 6/7 contribute to the E2 promoter activation and the E2 promoter elements that respond to these transactivators. We show that at eight hours following infection, E1A can transactivate the promoter about 21-fold whereas E4 6/7 can induce the promoter by only fivefold. DBP does not induce the promoter in the chromosomal context. Our mutational analysis suggests that the unique architecture of the E2-early promoter necessitates the concerted interaction of all three host transcription factors with their cognate recognition elements to form a stable and functional transcription complex. E1A mediated transactivation is dependent on this stable basal transcription complex and transactivation may involve simultaneous interaction of E1A with each of the three transcription factors present in the multicomponent basal transcription complex. The E4 6/7 protein can transactivate the E2-early promoter in the absence of ATF presumably by promoting the DNA binding capacity of transcription factor E2F and thereby stabilizing the basal transcription complex. We discuss some of the possible protein-protein interactions that may take place at the level of the multicomponent transcriptional complex at the E2-early promoter during transcriptional activation and the discrepancies that arise when a promoter is analyzed in infection versus transfection assays.

Record Date Created: 19960705

28/7/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08697339 96042030 PMID: 7589244

Cell type-specific expression of hnRNP proteins.

Kamma H; Portman D S; Dreyfuss G

Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki, Japan.

Experimental cell research (UNITED STATES) Nov 1995, 221 (1) p187-96 , ISSN 0014-4827 Journal Code: 0373226

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

HnRNP proteins are abundant nucleoplasmic pre-mRNA-binding proteins which have important roles in the biogenesis of mRNA. Although hnRNP proteins have been extensively characterized in cultured cell lines, little is known about their expression in animal tissues. Here, we have undertaken a systematic survey of the expression of major hnRNP proteins in mouse tissue using specific monoclonal antibodies. Immunohistochemical staining demonstrated that hnRNP proteins C, L, and U were localized to nuclei in all tissues examined. However, cytoplasmic expression of hnRNP A1 , D, F/H, and K was also detected in several tissues, suggesting that these proteins have roles in the cytoplasm as well as the nucleus. Importantly, the relative amounts of different hnRNP proteins varied among cell types. This was especially striking in neuronal and reproductive cells. In the brain, certain neuronal cell types contained more hnRNP proteins than glial cells, perhaps reflecting increased levels of neuronal transcription and RNA processing. In the ovary, oocytes contained exceptionally high

concentrations of hnRNP proteins as compared to follicular and stromal cells. In the testis, the expression of hnRNP proteins was generally high and was found to be tightly regulated during spermatogenesis. Specifically, hnRNP A1 was highly expressed only in early spermatogonia and absent in later stages. These findings demonstrate that hnRNP proteins do not exist in a fixed stoichiometry across different cell types. Furthermore, as the relative amounts of pre-mRNA-binding proteins (e.g., A1 and ASF/SF2) can affect alternative splicing patterns, the variations that we have observed could profoundly affect cell-specific gene expression .

Record Date Created: 19951214

28/7/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08565070 95322978 PMID: 7541274

Transcript analysis of CFTR nonsense mutations in lymphocytes and nasal epithelial cells from cystic fibrosis patients.

Will K; Dork T; Stuhrmann M; von der Hardt H; Ellemunter H; Tummler B; Schmidtke J

Abteilung fur Humangenetik, Medizinische Hochschule, Hannover, Germany.

Human mutation (UNITED STATES) 1995, 5 (3) p210-20, ISSN 1059-7794 Journal Code: 9215429

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The mutational effects at the mRNA level were investigated by RT-PCR analysis of nine different nonsense mutations (Q39X, E60X, R75X, G542X, L719X, Y1092X, R1162X, S1196X, W1282X) and one frameshift mutation (1078delT) within the CFTR gene. With the exception of mutation R1162X, reduced mRNA levels ranging from 30% to less than 5% of the wild type have been observed. In case of the R75X and E60X mutations, the mRNA reduction was accompanied by the appearance of atypical CFTR isoforms. Single exon 3 skipping, as well as joint exon 2 and 3 skipping, was observed in lymphocyte and nasal epithelial mRNA derived from R75X alleles. The analysis of mRNA transcribed from E60X alleles revealed skipping of exon 3 (lymphocytes and nasal epithelial cells) or skipping of exons 3 and 4 (nasal epithelial cells). With the exception of the E60X mutation, no obvious tissue-specific differences in the splicing pattern and ratios of mutation to wild-type transcripts were detected between lymphocytes and nasal epithelial cells. In addition to aberrant splicing , the reduction of transcripts is the most common effect of nonsense and frameshift mutations within the CFTR gene.

Record Date Created: 19950810

28/7/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08289546 95047327 PMID: 7958851

Regulation of tissue-specific P-element pre-mRNA splicing requires the RNA-binding protein PSI.

Siebel C W; Kanaar R; Rio D C

Department of Molecular and Cell Biology, University of California, Berkeley 94720.

Genes & development (UNITED STATES) Jul 15 1994, 8 (14) p1713-25, ISSN 0890-9369 Journal Code: 8711660
Contract/Grant No.: 5R01-HD28063-04; HD; NICHD
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Binding of a multiprotein complex to a 5' exon inhibitory element appears to repress splicing of the Drosophila P-element third intron (IVS3) in the soma. We have purified 97- and 50-kD proteins that interact specifically with the inhibitory element using RNA affinity chromatography. Antibodies specific for the 97-kD protein relieve inhibition of IVS3 splicing in somatic extracts, providing direct evidence that inhibition requires this protein, P-element somatic inhibitor (PSI). We identify the 50-kD protein as hrp48, a protein similar to the mammalian splicing factor hnRNP A1 , and show that hrp48 recognizes specific nucleotides in a pseudo-5' splice site within the inhibitory element. The results indicate that PSI is an alternative splicing factor that regulates tissue-specific splicing, probably through interactions with generally expressed factors such as hrp48.

Record Date Created: 19941201

28/7/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08277947 95037043 PMID: 7949729

Long-term survival of the exon 10 insertional cystic fibrosis mutant mouse is a consequence of low level residual wild-type Cftr gene expression.

Dorin J R; Stevenson B J; Fleming S; Alton E W; Dickinson P; Porteous D J
Medical Research Council Human Genetics, Unit, Western General Hospital, Edinburgh, UK.

Mammalian genome : official journal of the International Mammalian Genome Society (UNITED STATES) Aug 1994, 5 (8) p465-72, ISSN 0938-8990 Journal Code: 9100916
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Recently we have created a mouse model of cystic fibrosis (CF) by insertional gene targeting to exon 10. In common with CF subjects, this model displays a low incidence of meconium ileus. This contrasts strikingly with the very high level of fatal intestinal obstruction in the three other CF mouse models so far described. We investigate here the molecular basis of this difference in phenotype. We show that the partial duplication consequent upon insertional gene targeting allows exon skipping and aberrant splicing to produce normal Cftr mRNA, but at levels greatly reduced compared with wild-type mice. Furthermore, instead of the predicted mutant Cftr transcript, a novel mRNA is produced that utilizes cryptic splice sites in the disrupting plasmid sequence. However, we have previously shown that these mice display the ion transport defect characteristic of CF, and mutant animals can be distinguished from their normal littermates on this basis. Consistent with this, residual CFTR function has recently been observed for several "mild" mutations in CF individuals who display pancreatic sufficiency but still develop lung disease. We conclude that (i) residual wild-type mRNA in the exon 10

insertional mutant mouse ameliorates the severity of the intestinal phenotype observed in the absolute "null" CF mice, (ii) the presence of low-level residual wild-type Cftr mRNA does not correct the CF ion transport defect, and (iii) the long-term survival of this insertional mutant mouse provides the opportunity to address the factors important in development of lung disease.
Record Date Created: 19941228

28/7/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08247482 94367370 PMID: 8085156
Regulation of alternative splicing in vivo by overexpression of antagonistic splicing factors.
Caceres J F; Stamm S; Helfman D M; Krainer A R
Cold Spring Harbor Laboratory, NY 11724.
Science (UNITED STATES) Sep 16 1994, 265 (5179) p1706-9, ISSN 0036-8075 Journal Code: 0404511
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The opposing effects of SF2/ASF and heterogeneous nuclear ribonucleoprotein (hnRNP) A1 influence alternative splicing in vitro. SF2/ASF or hnRNP A1 complementary DNAs were transiently overexpressed in HeLa cells, and the effect on alternative splicing of several cotransfected reporter genes was measured. Increased expression of SF2/ASF activated proximal 5' splice sites, promoted inclusion of a neuron-specific exon, and prevented abnormal exon skipping. Increased expression of hnRNP A1 activated distal 5' splice sites. Therefore, variations in the intracellular levels of antagonistic splicing factors influence different modes of alternative splicing in vivo and may be a natural mechanism for tissue-specific or developmental regulation of gene expression .
Record Date Created: 19941012

28/7/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08235569 94375072 PMID: 7522211
Identification of eight mutations and three sequence variations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.
Ghanem N; Costes B; Girodon E; Martin J; Fanen P; Goossens M
Laboratoire de Genetique Moleculaire, INSERM U91, Hopital Henri Mondor, Creteil, France.
Genomics (UNITED STATES) May 15 1994, 21 (2) p434-6, ISSN 0888-7543 Journal Code: 8800135
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To determine cystic fibrosis (CF) defects in a sample of 224 non-delta F508 CF chromosomes, we used denaturing gradient gel multiplex analysis of CF transmembrane conductance regulator gene segments, a strategy based on blind exhaustive analysis rather than a search for known mutations. This

process allowed us to detect 11 novel variations comprising two nonsense mutations (Q890X and W1204X), a splice defect (405 + 4 A-->G), a frameshift (3293delA), four presumed missense mutations (S912L, H949Y, L1065P, Q1071P), and three sequence polymorphisms (R31C or 223 C/T, 3471 T/C, and T1220I or 3791 C/T). We describe these variations, together with the associated phenotype when defects on both CF chromosomes were identified.
Record Date Created: 19941020

28/7/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08174629 94309168 PMID: 8035503
Heterodimerization of the transcription factors E2F-1 and DP-1 is required for binding to the adenovirus E4 (ORF6/7) protein.
Helin K; Harlow E
Massachusetts General Hospital Cancer Center, Charlestown 02129.
Journal of virology (UNITED STATES) Aug 1994, 68 (8) p5027-35, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adenovirus infection leads to E1A-dependent activation of the transcription factor E2F. E2F has recently been identified in complexes with cellular proteins such as the retinoblastoma protein (pRB) and the two pRB family members p107 and p130. E1A dissociates E2F from these cellular proteins, and another viral protein, E4 (ORF6/7), can bind to E2F. The binding of E4 to E2F induces the formation of a stable DNA-binding complex containing the two proteins, and stimulation of the adenovirus E2 early promoter can occur. Recent studies have shown that E2F is the combined activity of several proteins, and we demonstrate here that heterodimerization of two of these proteins, E2F-1 and DP-1, is required for stable binding to E4. This complex is formed independently of DNA binding and requires the C-terminal 20 amino acids of E4. Furthermore, the binding is dependent on a region of E2F-1 between amino acids 284 and 358. This region of E2F-1 is conserved in E2F-2 and E2F-3, and deletion of this region drastically reduces the transcriptional activity of the molecule without affecting DP-1 binding, suggesting that this region of the E2F transcription factors is involved in regulating their activity. Our experiments also demonstrate that pRB binding to the E2F-1/DP-1 heterodimer prevents the formation of an E2F-1/DP-1/E4 complex.
Record Date Created: 19940815

28/7/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08006091 94136568 PMID: 7508188
CFTR and calcium-activated chloride currents in pancreatic duct cells of a transgenic CF mouse.
Gray M A; Winpenny J P; Porteous D J; Dorin J R; Argent B E
Department of Physiological Sciences, University Medical School, Newcastle upon Tyne, United Kingdom.
American journal of physiology (UNITED STATES) Jan 1994, 266 (1 Pt 1) pC213-21, ISSN 0002-9513 Journal Code: 0370511

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We have studied the cystic fibrosis transmembrane conductance regulator (CFTR) and calcium-activated chloride currents in pancreatic duct cells isolated from a transgenic cf/cf mouse created by targeted insertional mutagenesis. Adenosine 3',5'-cyclic monophosphate (cAMP)-activated CFTR chloride currents were detected in 78% (29/37) of wild-type cells, in 81% (35/43) of heterozygote cells, and in 61% (29/47) of homozygous cf/cf duct cells ($P > 0.05$, cf/cf vs. wild-type and heterozygote). The CFTR current density measured at membrane potentials of +/- 60 mV averaged 22-26 pA/pF in wild-type and heterozygote groups but only 13 pA/pF in cells derived from cf/cf animals ($P < 0.05$, cf/cf vs. wild-type and cf/cf vs. heterozygotes). In contrast, duct cells from animals of all three genotypic groups exhibited calcium-activated chloride currents that were of similar magnitude and up to 11-fold larger than the CFTR currents. We speculate that these transgenic insertional null mice do not develop the pancreatic pathology that occurs in cystic fibrosis patients because their duct cells contain 1) some wild-type CFTR generated by exon skipping and aberrant splicing and 2) a separate anion secretory pathway mediated by calcium-activated chloride channels.

Record Date Created: 19940310

28/7/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07868069 94004974 PMID: 7691356

Cystic fibrosis transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels.

Delaney S J; Rich D P; Thomson S A; Hargrave M R; Lovelock P K; Welsh M J ; Wainwright B J

Centre for Molecular Biology and Biotechnology, University of Queensland, Brisbane, Australia.

Nature genetics (UNITED STATES) Aug 1993, 4 (4) p426-31, ISSN 1061-4036 Journal Code: 9216904

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

In the human CFTR only the rare exon 4- splice variant is conserved in mice. We have discovered two novel murine variants, exon 5- and exon 11b+. The exon 5- variant represents up to 40% of mRNA in all CFTR -expressing tissues and leaves the reading frame intact. The exon 11b+ variant inserts a novel exon between exons 11 and 12 with expression restricted to the testis. Two variants of 11b have been found and both introduce premature stop codons. When we expressed human CFTR variants lacking either exon 5 or exon 9 in HeLa cells, they failed to generate cAMP-mediated chloride transport, due to defective intracellular processing. The lack of conservation of splice variants between species and the inability of the more abundant splice variants to generate protein that is correctly processed argue against a physiological role and may simply represent aberrant splicing that is tolerated by the cell and organism.

Record Date Created: 19931105

28/7/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07825092 93357737 PMID: 7689009
Abnormal mRNA splicing resulting from three different mutations in the CFTR gene.
Hull J; Shackleton S; Harris A
Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.
Human molecular genetics (ENGLAND) Jun 1993, 2 (6) p689-92, ISSN 0964-6906 Journal Code: 9208958
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Three different putative splicing mutations in the CFTR gene have been studied by analysing mRNA extracted from nasal epithelial cells harvested from patients with cystic fibrosis. Six patients were analysed, all of whom had classical symptoms of cystic fibrosis (CF). Two patients carried the 621 + 1G-->T mutation, 3 patients carried the 1717 - 1G-->A mutation and 1 patient carried the 1898 + 1G-->A mutation. All patients carried the delta F508 mutation on the other chromosome. Ten non-CF control subjects were also studied. The 621 + 1G-->T mutation resulted in activation of an alternative splice site within exon 4 in one patient and activation of this site or skipping of exon 4 in the other patient. The 1717 - 1G-->A mutation resulted in skipping of exon 11 in all 3 patients studied and the 1898 + 1G-->T mutation resulted in skipping of exon 12. These experiments demonstrate that these mutations do result in aberrant splicing of CFTR mRNA as predicted from the changes in genomic sequence.
Record Date Created: 19930921

28/7/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07415313 92347876 PMID: 1379210
Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (CFTR) coding regions and splice site junctions.
Farez B; Chanoz N; Vidaud M; Rosmond C; Martin J; Costes B; Blanco F; Goossens M
Laboratoire de Genetique Moleculaire, INSERM U91, Hopital Henri Mondor, Creteil, France.
Genomics (UNITED STATES) Jul 1992, 13 (3) p770-6, ISSN 0888-7543 Journal Code: 8800135
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The spectrum of cystic fibrosis (CF) mutations was determined in 105 patients by using denaturing gradient gel electrophoresis to screen the entire coding regions and adjacent cystic fibrosis transmembrane conductance regulator (CFTR) gene sequences. The nucleotide substitutions detected included 16 novel mutations, 11 previously described defects, and 11 nucleotide sequence polymorphisms. Among the novel mutations, 6 were of the missense type, 4 were nonsense mutations, 4 were frameshift defects , and 2 affected mRNA splicing . The mutations involved all the CFTR domains, including the R domain. Of the 61 non-delta F508 CF chromosomes

studied, mutations were found on 36 (59%), raising the proportion of CF alleles characterized in our patient cohort to 88%. Given the efficacy of the screening method used, the remaining uncharacterized mutations probably lie in DNA sequences outside the regions studied, e.g., upstream-promoter sequences, the large introns, or putative regulatory regions. Our results further document the highly heterogeneous nature of CF mutations and provide the information required for DNA-based genetic testing.
Record Date Created: 19920828

28/7/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07197595 92127594 PMID: 1531115
Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2.
Mayeda A; Krainer A R
Cold Spring Harbor Laboratory, New York, New York 11724-2208.
Cell (UNITED STATES) Jan 24 1992, 68 (2) p365-75, ISSN 0092-8674 Journal Code: 0413066
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
When messenger RNA precursors (pre-mRNAs) containing alternative 5' splice sites are spliced in vitro, the relative concentrations of the heterogeneous ribonucleoprotein (hnRNP) A1 and the essential splicing factor SF2 precisely determine which 5' splice site is selected. In general, an excess of hnRNP A1 favors distal 5' splice sites, whereas an excess of SF2 results in utilization of proximal 5' splice sites. The regulation of these antagonistic activities may play an important role in the tissue-specific and developmental control of gene expression by alternative splicing .
Record Date Created: 19920228

28/7/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06970029 91279453 PMID: 2057356
Tobacco nuclear gene for the 31 kd chloroplast ribonucleoprotein: genomic organization, sequence analysis and expression.
Li Y Q; Ye L Z; Sugita M; Sugiura M
Center for Gene Research, Nagoya University, Japan.
Nucleic acids research (ENGLAND) Jun 11 1991, 19 (11) p2987-91, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have previously identified three chloroplast ribonucleoproteins and characterized their cDNAs. Here we present the genomic organization, sequence and expression of one of their genes . The 31 kd ribonucleoprotein (cp31) from tobacco (Nicotiana sylvestris) chloroplasts is coded for by a single-copy nuclear gene. This gene was isolated and its sequence was determined. The gene contains four exons and three introns. The position of its first intron is conserved among the genes for the maize

abscisic acid induced glycine-rich protein, the human hnRNP A1 protein and cp31. The transcription start site was determined to be 168 bp upstream from the translational initiation codon in both leaf and root tissues. No alternatively spliced transcripts was detected, suggesting that a diversity of chloroplast ribonucleoproteins is generated probably by gene amplification rather than alternative splicing .
Record Date Created: 19910731

28/7/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06947783 91257832 PMID: 1710599
Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene.
Zielenski J; Bozon D; Kerem B ; Markiewicz D; Durie P; Rommens J M; Tsui L C
Department of Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.
Genomics (UNITED STATES) May 1991, 10 (1) p229-35, ISSN 0888-7543 Journal Code: 8800135
Contract/Grant No.: DK-34944-5; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Five different mutations have been identified in the gene causing cystic fibrosis (CF) through sequencing regions encompassing exons 1-8, including the 5' untranslated leader. Two of these apparent mutations are missense mutations, one in exon 3 (Gly to Glu at position 85; G85E) and another in exon 5 (Gly to Arg at 178; G178R), both causing significant changes in the corresponding amino acids in the encoded protein--cystic fibrosis transmembrane conductance regulator (CFTR). Two others affect the highly conserved RNA splice junction flanking the 3' end of exons 4 and 5 (621 + 1G----T, 711 + 1G----T), resulting in a probable splicing defect . The last mutation is a single-basepair deletion in exon 4, causing a frameshift. These five mutations account for the 9 of 31 non-delta F508 CF chromosomes in our Canadian CF family collection and they are not found in any of the normal chromosomes. Three of the mutations, 621 + 1G----T, 711 + 1G----T, and G85E, are found in the French-Canadian population, with 621 + 1G----T being the most abundant (5/7). There are two other sequence variations in the CFTR gene; one of them (129G----C) is located 4 nucleotides upstream of the proposed translation initiation codon and, although present only on CF chromosomes, it is not clear whether it is a disease-causing mutation; the other (R75Q) is most likely a sequence variation within the coding region.
Record Date Created: 19910718

28/7/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06173201 89259052 PMID: 2524598
The cellular transcription factor E2f requires viral E1A and E4 gene products for increased DNA-binding activity and functions to stimulate adenovirus E2A gene expression.
Babiss L E

Rockefeller University, New York, New York 10021-6399.
Journal of virology (UNITED STATES) Jun 1989, 63 (6) p2709-17,
ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: CA48707-01; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Whereas a wide variety of cellular proteins interact with the cis-regulatory elements of the adenovirus E1A and E2A genes, only the DNA-binding activity of the cellular E2f factor is modulated by viral early-gene expression. An analysis of cellular E2f protein levels and adenovirus early-gene expression in a panel of independently cloned virus-transformed rodent cell lines and in virus-infected rodent cells has established that both the E1A 289-amino-acid (289R) protein and a yet-to-be-defined E4 gene product are required for maximal E2f DNA-binding activity. To distinguish between the multiple roles the E1A protein could serve in this process, the E2f DNA-binding activity was determined in a virus-transformed cell line which contains a conditional-lethal mutation affecting the 289R protein. Since E4 gene expression was not altered by the incubation conditions, the observation of reduced cellular E2f activity at the nonpermissive temperature suggests a direct role for the E1A 289R protein in E2f activation. When a virus containing a deletion in the E4 gene was introduced into cell lines which can complement the E4 gene defect, a correlation between high cellular E2f levels and increased rates of E2A gene transcription was observed. A time course analysis of the viral infection revealed that E2f functions catalytically to stimulate viral E2A gene transcription. These observations have led to several hypotheses concerning possible mechanisms by which elevated E2A gene expression, which leads to cytotoxicity, might be avoided in the transformed cell.

Record Date Created: 19890627

28/7/36 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13761882 BIOSIS NO.: 200200390703
HnRNP - A1 binds directly to double stranded DNA in vitro within a 26 bp sequence.
AUTHOR: Donev Rossen M; Doneva Teodora A; Bowen W Richard; Sheer Denise(a)
AUTHOR ADDRESS: (a)Human Cytogenetics Laboratory, Cancer Research UK London Research Institute, Lincoln's Inn Field Laboratories, 44 Lincoln's Inn Fields, London, WC2A 3PX**UK E-Mail: Denise.Sheer@cancer.org.uk
JOURNAL: Molecular and Cellular Biochemistry 233 (1-2):p181-185 April, 2002
MEDIUM: print
ISSN: 0300-8177
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The heterogeneous nuclear ribonucleoprotein A1 (hnRNP - A1) is known as an RNA- and single-stranded DNA-binding protein involved in alternative splicing of mRNA, RNA transport and maintenance of chromosome telomere length. In this study we tested whether this protein could bind directly to double-stranded DNA (dsDNA). Using PCR

amplification of target DNA-sequences from human chromosome 11q13 followed by their incubation with hnRNP - A1 and atomic force microscopy (AFM) of the DNA/protein complexes, we found that this protein bound to DNA within a 36 bp sequence. These results were confirmed by electrophoretic mobility shift assay (EMSA). This sequence was found widely dispersed throughout the genome. There is no overlap between the 36 bp sequence and known target sequences in RNA for binding hnRNP - A1 .

28/7/37 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13740994 BIOSIS NO.: 200200369815
Tumor Necrosis Factor (alpha) is a negative regulator of protein kinase C(beta)II gene expression , Akt2 and SR protein dephosphorylation in vascular smooth muscle cells.
AUTHOR: Cooper Denise R(a); Watson James E; Chalfant Charles E; Chappell David S; Patel Niketa A; Mancu Dan; Mebert Konrad; Cheng Jin
AUTHOR ADDRESS: (a)J.A.Haley Veterans Hospital and University of South Florida, 13000 Bruce B. Downs Blvd., Tampa, FL, 33612**USA
JOURNAL: FASEB Journal 16 (5):pA917 March 22, 2002
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Insulin (INS) resistance contributes to a number of metabolic disorders including type 2 diabetes, hypertension and atherosclerosis. TNF(alpha) may mediate insulin resistance in vascular smooth muscle cells (VSMC) by inducing the dephosphorylation of key regulatory proteins in INS signaling and contribute to defects in energy homeostasis. We previously demonstrated that INS induces the alternative splicing of PKC(beta)II mRNA (J. Biol. Chem. 273:910, 1998); it increases the phosphorylation of serine/arginine rich (SR) proteins involved in the [illegible] PKC(beta)II is required for glucose uptake (Mol. Endo. 10:1273, 1996) and regulates cell proliferation. We examined effects of TNF(alpha) exposure on these events in A10 VSMC and found that TNF(alpha) pre-incubation blocked INS-induced alternative splicing of PKC(beta)II, and it also blocked INS-induced phosphorylation of SR proteins . This effect was mimicked by C6-ceramide, a known mediator of TNF(alpha) signaling. Both C6 and TNF(alpha) maintained Akt2, a kinase involved in the alternative splicing of PKC(beta)II, in an inactive dephosphorylated state in the presence of INS. Our results indicate that TNF(alpha) may induce INS resistance in VSMC by maintaining Akt2 and SR proteins in inactive, dephosphorylated states that block PKC(beta)II splicing.

28/7/38 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13622313 BIOSIS NO.: 200200251134

Alternative splicing : Multiple control mechanisms and involvement in human disease .
AUTHOR: Caceres Javier F(a); Kornblihtt Alberto R
AUTHOR ADDRESS: (a)MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU**UK E-Mail: Javier.Caceres@hgu.mrc.ac.uk, ark@bg.fcen.uba.ar
JOURNAL: Trends in Genetics 18 (4):p186-193 April, 2002
MEDIUM: print
ISSN: 0168-9525
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Alternative splicing is an important mechanism for controlling gene expression . It allows large proteomic complexity from a limited number of genes. An interplay of cis-acting sequences and trans-acting factors modulates the splicing of regulated exons. Here, we discuss the roles of the SR and hnRNP families of proteins in this process. We also focus on the role of the transcriptional machinery in the regulation of alternative splicing , and on those alterations of alternative splicing that lead to human disease .

28/7/39 (Item 4 from file: 5)

13581008 BIOSIS NO.: 200200209829
Characterization of a second hnRNP A/B-dependent exonic splicing silencer in protein 4.1R exon 16.
AUTHOR: Hou Victor C(a); Gee Sherry L(a); Wu Michael; Turck Chris W; Koury Mark; Krainer Adrian R; Mayeda Akila; Conboy John G(a)
AUTHOR ADDRESS: (a)Lawrence Berkeley National Laboratory, Berkeley, CA**USA
JOURNAL: Blood 98 (11 Part 1):p436a November 16, 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971
LANGUAGE: English

ABSTRACT: A fundamental switch in protein 4.1R gene expression during erythropoiesis is mediated through the alternative pre-mRNA splicing of exon 16 (E16), which encodes a critical region of the spectrin-actin binding domain essential for normal membrane mechanical properties. Whereas the splicing machinery in early erythroid progenitors skips E16, most of the 4.1R mRNA in later progenitors includes E16. A major goal of these studies is to identify both the regulatory elements in 4.1 pre-mRNA, and the relevant splicing factor proteins, that control E16 splicing. In previous studies we identified a conserved sequence in E16 that behaves as a splicing silencer element and binds to a known splicing inhibitory protein, hnRNP A1 . We also reported that the 5' purine-rich element (PRE16) of E16 resembled splicing enhancer elements characterized in other pre-mRNAs. Here we show that PRE16 actually behaves in the context of 4.1R pre-mRNA as a second splicing silencer element within E16, and that both PRE16 and CE16 silencer elements are required for proper regulation of E16 splicing. Model 4.1R pre-mRNAs

containing wild type or mutated E16 sequences were subject to splicing assays in vitro or in transfected HeLa cells, and the spliced mRNA products were analyzed by RT/PCR. Substitution of PRE16 with neutral RNA lacking known splicing elements greatly activated E16 inclusion, consistent with the localization of a silencer element in PRE16. Further supporting this interpretation, excess PRE16 RNA was able to titrate the silencer activity from nuclear extracts, allowing more efficient E16 inclusion upon splicing of the model 4.1R pre-mRNA. Mass spectrometry and Western blot analysis showed that, analogous to CE16, one of the proteins bound to PRE16 was hnRNP A1 . Recombinant full length A1 protein was able to restore silencing activity to nuclear extracts depleted of PRE16-binding proteins in a concentration-dependent manner, whereas mutated A1 proteins were inactive. Further analysis using gel mobility shift assays showed that both PRE16 and CE16 can bind directly to hnRNP A1 . However, splicing assays with mutated pre-mRNAs demonstrated that the composite silencer containing both elements is much stronger than either element alone. Together these results suggest exon 16 splicing may be under negative control by hnRNP A1 protein, and that developmental regulation of splicing could be effected by changes in A1 concentration. Indeed, we have shown using a mouse erythropoiesis model system that there is a striking temporal correlation between the down-regulated expression of hnRNP A/B proteins during erythroid differentiation and the increased inclusion of E16 in endogenous 4.1R pre-mRNA. This effect appears specific for hnRNP A/B proteins, since other splicing factors of the hnRNP family, including hnRNP H and PTB, exhibit different expression patterns in differentiating erythoblasts. These findings demonstrate that natural developmental changes in hnRNP A/B protein expression can effect physiologically important switches in pre-mRNA splicing through interactions with silencer elements within E16.

28/7/40 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13524239 BIOSIS NO.: 200200153060
p210BCR/ABL-induced alteration of mRNA splicing as a potential mechanism of CML pathogenesis.

AUTHOR ADDRESS: (a)Stem Cell Institute, University of Minnesota Cancer Center, Minneapolis, MN**USA
JOURNAL: Blood 98 (11 Part 1):p144a November 16, 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Chronic Myelogenous Leukemia (CML) is a malignancy of the human hematopoietic stem cell (HSC) characterized by presence of the BCR/ABL gene and its product, p210BCR/ABL. The exact mechanism(s) underlying p210BCR/ABL-mediated transformation is not totally understood. Pre-mRNA processing is an important step in the transition from DNA to protein, wherein exons likely require different groups of trans-acting factors and cis-acting elements for their proper union. Although alternative splicing of mRNA is a critical determinant of a cell's protein

repertoire, it has never been implicated in CML pathogenesis. Subtractive hybridization techniques comparing MSCV-IRES-GFP and MSCV-p210BCR/ABL-IRES-GFP transduced cord blood (CB) CD34+ cells identified multiple genes involved in pre-mRNA splicing in BCR/ABL-positive cells, including the SR - protein kinase 1 (SRPK1) and RNA Helicase II/Gu. Northern blot, quantitative real-time PCR, and Western blot analyses have confirmed increased expression of these genes and their products in primary CML versus normal CD34+ cells and p210BCR/ABL versus GFP-transduced CB CD34+ cells. Moreover, the phosphorylation of SRPK1 substrates, such as SR proteins , which are critical components of the spliceosome complex, is increased in p210BCR/ABL-containing cells. Intriguingly, previous experiments in our lab investigating the abnormal adhesion and migration of BCR/ABL-positive cells characterized alternatively splicing of the non-receptor tyrosine kinase gene, PYK2, whose product is intimately involved in betal-integrin signaling. In p210BCR/ABL-positive cells, the ratio of full-length Pyk2 versus the PYK2 isoform normally predominant in hematopoietic cells, Pyk2H, is increased. Alternative splicing of PYK2 is directly influenced by p210BCR/ABL as indicated by the significantly higher ratio of Pyk2/Pyk2H in primary CML versus normal CD34+ cells and p210BCR/ABL versus GFP-transduced CB CD34+ cells. Moreover, treatment of primary CML CD34+ cells or p210BCR/ABL-transduced CB CD34+ cells with the Abl-specific tyrosine kinase inhibitor, STI571, normalized the Pyk2/Pyk2H ratio. This STI571-induced reversion to normal PYK2 gene splicing correlated with decreased expression of the splicing proteins SRPK1 and RNA Helicase II/Gu, and reduced phosphorylation of the SR proteins . Reversible phosphorylation of SR proteins is critical for spliceosome assembly and subsequent splicing, and SRPK1 overexpression inhibits pre-mRNA splicing (Genes Dev 1996 10:1569). Therefore, experiments are in progress in which SRPK1 is overexpressed in normal CB CD34+ cells, by MSCV-based retroviral transduction, to determine whether the BCR/ABL-induced alteration of PYK2 mRNA splicing is mediated solely by the elevation of SRPK1 expression/activity, and whether these changes in splicing contribute to the abnormal adhesion and migration characteristics observed in CML.

28/7/41 (Item 6 from file: 5)

13517581 BIOSIS NO.: 200200146402

Qualitative and quantitative analysis of mRNA associated with four putative splicing mutations (621+3AfwdarwG, 2751+2TfwdarwA, 296+1GfwdarwC, 1717-9TfwdarwC-D565G) and one nonsense mutation (E822X) in the CFTR gene.

AUTHOR: Tzetis Maria; Efthymiadou Alexandra; Doudounakis Stavros; Kanavakis Emmanuel(a)

AUTHOR ADDRESS: (a)Department of Medical Genetics, Athens University, "Aghia Sophia" Children's Hospital, Thivon and Livadias, Athens, 11527** Greece E-Mail: ekanavak@cc.uoa.gr

JOURNAL: Human Genetics 109 (6):p592-601 December, 2001

MEDIUM: print

ISSN: 0340-6717

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The effects of four splicing mutations and one nonsense mutation on cystic fibrosis transmembrane conductance regulator (CFTR) gene expression were investigated by reverse transcription-polymerase chain reaction analysis of mRNA extracted from nasal epithelial cells harvested from patients harbouring the mutations. We studied four subjects with 621+3AfwdarwG, two with 2751+2TfwdarwA, one with 296+1GfwdarwC, two with 1717-9TfwdarwC-D565G and seven with E822X and compared the results with CFTR mRNA from normal subjects. Our results showed that mutations 621+3AfwdarwG, 2751+2TfwdarwA, and 296+1GfwdarwC, which disrupt the 5' splice donor sites of introns 4, 14a, and 2, respectively, and 1717-9TfwdarwC-D565G, which possibly disrupts the exonic splicing enhancer sequences of exon 12 (owing to the missense mutation in cis), lead to the production of aberrantly spliced mRNA in nasal epithelial cells. Three of the splicing mutations (621+3AfwdarwG, 2751+2TfwdarwA, and 296+1GfwdarwC) result in severe deficiency of normal CFTR mRNA and severe phenotype in the patients. This information is especially useful for mutation 621+3AfwdarwG, which is found in other populations as well, and was initially reported as a polymorphism. The complex allele 1717-9TfwdarwC-D565G results in aberrant splicing of CFTR mRNA with production of transcripts lacking exon 12 (major product), with minor amounts of transcripts revealing joint exon 11 and 12 skipping. Nonsense mutation E822X results in a severe reduction in mRNA levels to about 6% of wild type. Patients with the mutation have a severe clinical phenotype, with both the pancreatic and the pulmonary function affected.

28/7/42 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13394502 BIOSIS NO.: 200200023323
The effect of cellular and viral splicing factors on the level of normal CFTR RNA.
AUTHOR: Nissim-Rafinia M(a); Kerem B(a)
AUTHOR ADDRESS: (a)Dept Genetics, Hebrew Univ, Jerusalem**Israel
JOURNAL: American Journal of Human Genetics 69 (4 Supplement):p650 October, 2001
CONFERENCE/MEETING: 51st Annual Meeting of the American Society of Human Genetics San Diego, California, USA October 12-16, 2001
ISSN: 0002-9297
RECORD TYPE: Citation
LANGUAGE: English

28/7/43 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13202312 BIOSIS NO.: 200100409461
Cloning of a gene (SR-A1), encoding for a new member of the human Ser/Arg-rich family of pre-mRNA splicing factors: Overexpression in aggressive ovarian cancer.
AUTHOR: Scorilas A; Kyriakopoulou L; Katsaros D; Diamandis E P(a)
AUTHOR ADDRESS: (a)Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, M5G 1X5: Scorilas@netscape.net**Canada

JOURNAL: British Journal of Cancer 85 (2):p190-198 20 July, 2001
MEDIUM: print
ISSN: 0007-0920
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: By using the positional cloning gene approach, we were able to identify a novel gene encoding for a serine/arginine-rich protein, which appears to be the human homologue of the rat A1 gene. We named this new gene SR-A1. Members of the SR family of proteins have been shown to interact with the C-terminal domain (CTD) of the large subunit of RNA polymerase II and participate in pre-mRNA splicing. We have localized the SR-A1 gene between the known genes IRF3 and RRAS on chromosome 19q13.3. The novel gene spans 16.7 kb of genomic sequence and it is formed of 11 exons and 10 intervening introns. The SR-A1 protein is composed of 1312 amino acids, with a molecular mass of 139.3 kDa and a theoretical isoelectric point of 9.31. The SR-A1 protein contains an SR-rich domain as well as a CTD-binding domain present only in a subset of SR - proteins . Through interactions with the pre-mRNA and the CTD domain of the Polymerase II, SR proteins have been shown to regulate alternative splicing . The SR-A1 gene is expressed in all tissues tested, with highest levels found in fetal brain and fetal liver. Our data suggest that this gene is overexpressed in a subset of ovarian cancers which are clinically more aggressive. Studies with the steroid hormone receptor-positive breast and prostate carcinoma cell lines ZR-75-1, BT-474 and LNCaP, respectively, suggest that SR-A1 is constitutively expressed. Furthermore, the mRNA of the SR-A1 gene in these cell lines appears to increase by estrogens, androgens and glucocorticoids, and to a lesser extend by progestins.

13106865 BIOSIS NO.: 200100314014

alternative splicing of protein 4.1R exon 16 through an interaction with an exonic splicing silencer.

AUTHOR: Hou Victor C(a); Gee Sherry L(a); Wu Michael; Turck Christoph W; Mayeda Akila; Conboy John G(a)
AUTHOR ADDRESS: (a)Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA**USA

JOURNAL: Blood 96 (11 Part 1):p441a November 16, 2000
MEDIUM: print
CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000
SPONSOR: American Society of Hematology
ISSN: 0006-4971
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: A fundamental switch in protein 4.1R gene expression during erythropoiesis is mediated at the level of alternative pre-mRNA

splicing of exon 16 (E16), which encodes a critical region of the spectrin-actin binding domain essential for normal membrane mechanical properties. Several lines of evidence suggest that one of the molecular determinants that regulates splicing of E16 is a 42 nt evolutionarily conserved sequence in E16 that functions as an exonic splicing silencer (ESS16). In model 4.1R pre-mRNA constructs spliced in vitro with HeLa cell nuclear extracts, or in vivo in microinjected Xenopus oocyte nuclei, mutation of ESS16 or replacement of ESS16 with heterologous RNA sequence leads to more efficient inclusion of E16. Conversely, insertion of ESS16 into heterologous pre-mRNA constructs greatly decreases splicing of an adjacent upstream intron. These experiments support the hypothesis that native ESS16 binds to nuclear factor(s) that inhibit inclusion of E16. A biotinylated ESS16 RNA affinity probe incubated in HeLa cell nuclear extract binds to several proteins, including a apprx34 kD protein confirmed by Western blotting and nanospray mass spectrometry to be hnRNP A1 , a known nuclear RNA-binding protein/ alternative splicing factor. In functional splicing assays, addition of excess recombinant hnRNP A1 to HeLa cell nuclear extract results in almost complete exclusion of E16. In contrast, partial depletion of hnRNP A1 from nuclear extract greatly improves E16 inclusion, while add-back of wild-type hnRNP A1 , but not of an inactive mutant, restores inhibition of E16 inclusion in a concentration-dependent manner. These results indicate that the interaction of hnRNP A1 with ESS16 specifically blocks inclusion of E16. Experiments with mouse erythroleukemia cells demonstrated that DMSO-induced differentiation is accompanied by an apprxtwo-fold decrease in hnRNP A1 protein levels coordinated with an up to five-fold increase of 4.1R protein isoforms containing the peptide encoded by E16. These results are consistent with a model in which developmentally-regulated expression of hnRNP A1 in erythropoiesis can regulate the functionally-critical splicing switch of E16 through interactions with the ESS16 element in protein 4.1 pre-mRNA.

28/7/45 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11899677 BIOSIS NO.: 199900145786
Control of 5' splice site choice in vitro by hnRNP
AUTHOR: Bai Yidong; Lee Diana; Yu Tongde; Chasin Lawrence A(a)
AUTHOR ADDRESS: (a)Dep. Biological Sciences, Columbia Univ., New York, NY 10027**USA
JOURNAL: Nucleic Acids Research 27 (4):p1126-1134 Feb. 15, 1999
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The constitutive splicing factor ASF/SF2 has been shown to affect the choice between alternative splice sites by favoring the proximal as opposed to the distal choice. HnRNP A1 antagonizes ASF/SF2 by promoting the distal choice for competing 5' splice sites. We have tested the in vivo effects of these proteins on alternative 3' splice site choices. Cotransfection of a dihydrofolate reductase-calcitonin chimeric construct together with a plasmid specifying the SR protein ASF/SF2 into cells of several mammalian lines increased use of a proximal 3' splice site, resulting in the inclusion of a terminal calcitonin exon. This

stimulation of 3' proximal splicing was antagonized by cotransfection with an hnRNP A1 plasmid. This effect of hnRNP A1 in promoting distal splicing was also seen in an hnRNP A1 -deficient MEL cell line. A similar effect of hnRNP A1 was demonstrated with mutant hamster adenine phosphoribosyltransferase (aprt) transcripts that are normally constitutively spliced, suggesting that hnRNP A1 may be a general inhibitor of proximal splicing. Intron size also influenced splice site choice in mutant aprt transcripts, with larger introns favoring proximal splicing. These results support the idea that the ratios of particular but general splicing factors and hnRNPs play a role in alternative splicing .

28/7/46 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11330668 BIOSIS NO.: 199800112000
Antisense-mediated correction of aberrant splicing in a stable cell line expressing a CFTR mini-gene harboring the CF-causing mutation, 3849+10kb C greater than T.
AUTHOR: Friedman K J(a); Wang Y; Silverman L M; Knowles M R; Kole R
AUTHOR ADDRESS: (a)Curriculum Genetics, Univ. North Carolina, Chapel Hill, NC**USA
JOURNAL: American Journal of Human Genetics 61 (4 SUPPL.):pA355 Oct., 1997
CONFERENCE/MEETING: 47th Annual Meeting of the American Society of Human Genetics Baltimore, Maryland, USA October 28-November 1, 1997
ISSN: 0002-9297
RECORD TYPE: Citation
LANGUAGE: English

28/7/47 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10891779 BIOSIS NO.: 199799512924
A highly stable duplex structure sequesters the 5' splice region of hnRNP A1 alternative exon 7B
AUTHOR: Blanchette Marco; Chabot Benoit(a)
AUTHOR ADDRESS: (a)Dep. Microbiol. Infect., Fac. Med., Univ. Sherbrooke, 3001 12e Avenue Nord, Sherbrooke, PQ J1H 5**Canada
JOURNAL: RNA (New York) 3 (4):p405-419 1997
ISSN: 1355-8382
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Exon 7B in the hnRNP A1 pre-mRNA is alternatively spliced to yield A1 and A1-B, two proteins that diff er in their ability to modulate 5' splice site selection. Sequencing the murine intron downstream of axon 7B revealed the existence of several regions of similarity to the corresponding human intron. In vitro splicing assays indicate that an 84-nt region (CE610) decreases splicing to the proximal 5' splice site in a pre-mRNA carrying the 5' splice sites of exon 7 and 7B. In vivo, the CE610 element promotes exon 7B skipping in pre-mRNAs expressed from a mini- gene containing the hnRNP A1 alternative splicing unit. Using oligonucleotide-targeted RNase H cleavage assays, we provide

support for the existence of highly stable base pairing interactions between CE610 and the 5' splice site region of exon 7B. Duplex formation occurs in naked pre-mRNA, resists incubation in splicing extracts, and is associated with a reduction in the assembly of U1 snRNP-dependent complexes to the 5' splice site of exon 7B. Our results demonstrate that pre-mRNA secondary structure plays an important role in promoting exon 7B skipping in the A1 pre-mRNA.

28/7/48 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

11111817 EMBASE No: 2001130171
Spatio-temporal expression of the Trans-acting splicing factors SF2/ASF and heterogeneous ribonuclear proteins A1/A1SUPB in the myometrium of the pregnant human uterus: A molecular mechanism for regulating regional protein isoform expression in Vivo

Pollard A.J.; Sparey C.; Robson S.C.; Krainer A.R.; Europe-Finner G.N.
Dr. A.J. Pollard, Department of Obstetrics, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP United Kingdom
AUTHOR EMAIL: a.j.pollard@ncl.ac.uk

Journal of Clinical Endocrinology and Metabolism (J. CLIN. ENDOCRINOL. METAB.) (United States) 2000, 85/5 (1928-1936)
CODEN: JCEMA ISSN: 0021-972X
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 47

Many of the human myometrial proteins associated with uterine quiescence and the switch to coordinated contractions at the onset of labor exist as alternatively spliced isoforms. There is now extensive evidence to indicate that the nuclear concentrations of the trans-acting splicing regulators SF2/ASF and hnRNP A1 /A1SUPB are fundamental in regulating the expression of specific protein isoforms derived from alternative splicing of single precursor messenger ribonucleic acid transcripts. The question thus arose as to whether these factors were also involved in regulating the expression of specific myometrial protein isoforms in different uterine regions during human gestation and parturition. SF2/ASF and hnRNP A1 /A1SUPB expression was therefore determined in paired upper (corpus) and lower segment myometrial samples taken from individual women at term/during spontaneous labor and compared with nonpregnant control samples using specific monoclonal antibodies. We report that SF2/ASF levels were substantially increased in the lower uterine region, and this was associated with a parallel decrease in levels of hnRNP A1 /A1SUPB during gestation. Conversely, the opposite pattern was observed within the upper uterine region during pregnancy, where hnRNP A1 /A1SUPB was significantly up-regulated and SF2/ASF levels were much less than those found in the lower uterine segment. The differential expression of hnRNP A1 /A1SUPB and SF2/ASF in the upper and lower uterine segments may have a primary role in defining the formation of specific myometrial protein species associated with the known contractile and relaxatory properties of these regions before and during parturition.
?logoff hold

Kam 09/871,809

=> d his 1

(FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 15:04:25 ON 30 APR 2003)

L29 73 DUP REM L28 (114 DUPLICATES REMOVED)

=> d que l29

L3 3926 SEA (ABERRANT OR ABNORMAL)(3A) SPLIC?
L4 4966 SEA EXON(3A) SKIP?
L5 175 SEA INTRON(3A) EXCLU?
L6 58172 SEA ALTERNATIV?(3A) SPLIC?
L7 488 SEA ERRO?(5A) SPLIC?
L8 8805 SEA (L3 OR L4 OR L5) OR L7
L9 1977 SEA L8 AND L6
L11 23 SEA L9 AND E4?
L12 70 SEA L9 AND A1
L18 229 SEA (L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17)
L19 6768 SEA TRANSITION(3A) MUTA?
L20 12 SEA L9 AND L19
L21 240 SEA L18 OR L20
L22 5628 SEA C(3A) FWDARW(3A) T
L23 10 SEA L22 AND L9
L24 246 SEA L21 OR L23
L25 30 SEA L24 AND NORMAL?
L26 24 SEA L24 AND (TREAT? OR THERAP? OR ADMINIST?)
L27 158 SEA L24 NOT PY>1999
L28 187 SEA L27 OR L25 OR L26
L29 73 DUP REM L28 (114 DUPLICATES REMOVED)

=> d ibib abs 129 1-73

L29 ANSWER 1 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2003:175210 SCISEARCH
THE GENUINE ARTICLE: 646ZY
TITLE: **Abnormal splicing** of SHP-1 protein tyrosine phosphatase in human T cells: Implications for lymphomagenesis
AUTHOR: Ma X Z; Jin T R; Sakac D; Fahim S; Zhang X; Katsman Y; Bali M; Branch D R (Reprint)
CORPORATE SOURCE: Toronto Ctr, Canadian Blood Serv, 67 Coll St, Toronto, ON M5G 2M1, Canada (Reprint); Toronto Ctr, Canadian Blood Serv, Toronto, ON M5G 2M1, Canada; Univ Toronto, Inst Med Sci, Toronto, ON M5S 1A1, Canada; Univ Toronto, Dept Med, Toronto, ON, Canada; Toronto Gen Res Inst, Div Cell & Mol Biol, Toronto, ON, Canada
COUNTRY OF AUTHOR: Canada
SOURCE: EXPERIMENTAL HEMATOLOGY, (FEB 2003) Vol. 31, No. 2, pp. 131-142.
Publisher: ELSEVIER SCIENCE INC, 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA.
ISSN: 0301-472X.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Objective. SHP-1 protein tyrosine phosphatase has been implicated in suppressing B-lymphocyte and myeloid cell malignancies; however, there are little data on this role of SHP-1 in T-lymphocyte malignancies. We

Search completed by David Schreiber 308-4292

examined malignant human T cells to identify any abnormalities of SHP-1 that would support a role for this molecule in suppressing T lymphontagenesis.

Materials and Methods. Human T-lymphocyte cell lines and primary blood cells were used to examine the expression of SHP-1 mRNA and protein. Reverse transcriptase polymerase chain reaction was used to amplify particular portions of the SHP-1 mRNA for cloning and sequencing. Gene transfer was used to examine the effects of SHP-1 on cell growth and morphology. Glutathione S-transferase (GST) fusion proteins were generated and used to determine SHP-1-associated proteins.

Results. Leukemia- and lymphoma-derived cell lines were identified that did not express SHP-1 protein. Examination of the mRNA from these and other T-cell lines, and from **normal** peripheral blood mononuclear cells (PBMCs), revealed three distinct transcripts by restriction enzymes, reverse transcriptase polymerase chain reaction, and Southern blot analysis. In addition to the expected wild-type transcript, two novel transcripts were identified. One was a deletion transcript found only in Jurkat leukemia-derived cells, predicted to encode for a 7-kDa protein containing most of the amino-terminal SH2 domain. The second contained an 88-nucleotide insert that is the unspliced second intron resulting in a frame shift and the formation of a noncoding transcript. This mRNA was found in all cells examined but was the only transcript detected in the cell lines lacking SHP-1 protein. Expressing wild-type SHP-1 in these cell lines resulted in a change in the morphology of the cells with a concomitant decrease in their growth. GST fusion constructs showed the 7-kDa variant able to associate with an identical array of proteins as wild-type SHP-1, suggesting that it could compete with the wild-type SHP-1 for substrates. This protein was detectable in the cell line expressing its corresponding mRNA and was able to induce significant changes in cell morphology when transfected into a cell line expressing wild-type SHP-1; however, it did not induce any changes in cell growth.

Conclusions. These data are the first to show the existence of multiple transcripts of SHP-1 in human transformed T lymphocytes and **normal** PBMCs and supports previous work showing that alternate forms of SHP-1 mRNA are a common finding in other cells. We also show the lack of splicing out of an intron as a novel mechanism of regulation of SHP-1 protein expression in both **normal** and transformed T cells. Moreover, we provide the first evidence showing a protein product detectable in cells that is translated from an **alternatively spliced** form of SHP-1 mRNA, a variant truncated SHP-1 protein having potential biologic relevance. This report provides evidence supporting the concept that SHP-1 can negatively regulate growth of malignant human T cells and that lack of SHP-1 protein or function may be associated with lymphomagenesis. (C) 2003 International Society for Experimental Hematology.

L29 ANSWER 2 OF 73 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2003045519 MEDLINE
DOCUMENT NUMBER: 22442694 PubMed ID: 12524529
TITLE: Correction of disease-associated **exon skipping** by synthetic **exon**-specific activators.
AUTHOR: Cartegni Luca; Krainer Adrian R
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724, USA.
SOURCE: NATURE STRUCTURAL BIOLOGY, (2003 Feb) 10 (2) 120-5. Journal code: 9421566. ISSN: 1072-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

Search completed by David Schreiber 308-4292

Kam 09/871,809

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20030130
Last Updated on STN: 20030226
Entered Medline: 20030225

AB Differential exon use is a hallmark of **alternative splicing**, a prevalent mechanism for generating protein isoform diversity. Many disease-associated mutations also affect pre-mRNA splicing, usually causing inappropriate **exon skipping**. **SR** proteins are essential splicing factors that recognize exonic splicing enhancers and drive exon inclusion. To emulate this function of **SR** proteins, we designed small chimeric effectors comprising a minimal synthetic RS domain covalently linked to an antisense moiety that targets an exon by Watson-Crick base pairing. Here we show that such synthetic effectors can mimic the functions of **SR** proteins and specifically restore wild type splicing when directed to defective BRCA1 or SMN2 pre-mRNA transcripts. This general approach can be used as a tool to investigate **splicing** mechanisms and modulate **alternative splicing** of specific genes, and as a **therapeutic** strategy to correct splicing defects responsible for numerous diseases.

L29 ANSWER 3 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:368628 HCAPLUS
DOCUMENT NUMBER: 136:381346
TITLE: Regulation of survival motor neuron exon 7 and BRCA1 exon 18 splicing using peptide nucleic acid molecules for **treatment** of diseases
INVENTOR(S): Krainer, Adrian R.; Cartegni, Luca
PATENT ASSIGNEE(S): Cold Spring Harbor Laboratory, USA
SOURCE: PCT Int. Appl., 76 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002038738	A2	20020516	WO 2001-US47523	20011109
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002032536	A5	20020521	AU 2002-32536	20011109

PRIORITY APPLN. INFO.: US 2000-304182P P 20001109
WO 2001-US47523 W 20011109

AB The present invention provides a chimeric mol. including a covalently-bonded base-pairing segment that binds specifically to a single-stranded nucleic acid mol. like a pre-mRNA transcript and a moiety that modulates splicing or translation. A spacer may be present between the base-pairing region and said moiety of about 1-20 amino acids and may contain at least one glycine residue. The moiety may be a protein or a second nucleic acid. The base-pairing region comprises a non-sugar

backbone like peptide-nucleic acids or morpholino groups or a 2' O-Me ribose group backbone. Furthermore, the chimeric mol. may have a branched structure and the base-pairing segment may be 6-50 bases. A protein moiety in the chimeric mol. may be 5-50 amino acids and may comprise a translation or splicing activation domain. Furthermore, the protein domain may contain dipeptide repeats. The second nucleic acid in a chimeric mol. may be an endogenous splicing protein binding site. In a specific embodiment, the chimeric mol. may bind the SMN2 exon 7 pre-mRNA transcript between 0-300 residues away from the splice site and modulate splicing. Modulation of splicing promotes inclusion of target exon in mRNA due to mutation in exonic splicing enhancer element of said target exon and thereby these chimeric mols. may be used in **treatment** of spinal muscular atrophy in utero. In another embodiment, a peptide nucleic acid peptide was effective at promoting exon 18 inclusion with pre-mRNA harboring a nonsense mutation in a patient. The invention also provides a chimeric mol. including a base-pairing segment that binds specifically to a double-stranded nucleic acid mol. and a peptide that modulates transcription, wherein the peptide comprises up to about one hundred amino acid residues.

L29 ANSWER 4 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:123508 HCAPLUS
DOCUMENT NUMBER: 136:162403
TITLE: Control of aberrant gene expression by **alternative splicing** factor
INVENTOR(S): Kerem, Batsheva
PATENT ASSIGNEE(S): Yissum Research Development Company of the Hebrew University of Jerusalem, Israel
SOURCE: U.S. Pat. Appl. Publ., 15 pp., Cont.-in-part of U.S. Ser. No. 421,891, abandoned.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002018768	A1	20020214	US 2001-871809	20010604
PRIORITY APPLN. INFO.:			US 1999-421891 B2	19991021

AB The invention concerns a method for **treating** various genetic [illegible] factors which can modulate **alternative splicing**. The method of the present invention is esp. suitable for the **treatment** of **cystic fibrosis**.

L29 ANSWER 5 OF 73 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2002382703 MEDLINE
DOCUMENT NUMBER: 22126689 PubMed ID: 12130684
TITLE: Alternative processing of the human FMO6 gene renders transcripts incapable of encoding a functional flavin-containing monooxygenase.
AUTHOR: Hines Ronald N; Hopp Kathleen A; Franco Jose; Saeian Kia; Begun Frank P
CORPORATE SOURCE: Department of Pediatrics, Birth Defects Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53226-4801, USA.. rhines@mail.mcw.edu
CONTRACT NUMBER: CA53106 (NCI)
SOURCE: MOLECULAR PHARMACOLOGY, (2002 Aug) 62 (2) 320-5.

Search completed by David Schreiber 308-4292

Journal code: 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AL021026
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020720
Last Updated on STN: 20020823
Entered Medline: 20020805

AB The flavin-containing monooxygenases (FMOs) are a family of five microsomal enzymes important for the oxidative metabolism of environmental toxicants, natural products, and **therapeutics**. With the exception of FMO5, the FMO are encoded within a single gene cluster on human chromosome 1q23-25. As part of the human genome effort, an FMO-like gene, FMO6, was identified between FMO3 and FMO2 (GenBank accession no. AL021026). Sequence analysis of this putative FMO family member revealed nothing that would a priori argue against a functional gene and encoded protein. When FMO6 expression was examined by reverse transcriptase coupled polymerase chain reaction DNA amplification, transcripts were identified in 8 of 11 human liver samples, but 0 of 4 kidney biopsy samples. However, in all cases, the observed transcripts were shorter than predicted. Sequence analysis revealed **skipping** of **exon** 4, exons 3 and 4, and/or the use of **alternative splice** donor or acceptor sites in introns 3, 4, 6, and 8, resulting in nine unique transcripts. Based on an analysis of possible open reading frames, none of these transcripts would encode a functional FMO enzyme. Taking advantage of the high sequence identity between FMO3 and FMO6, it is posited that the loss of binding sites for the **serine-arginine-rich** splicing factor protein family within exons 3 and 4 contributes to the **exon skipping** events, although the most commonly observed **alternative splice** event results from a 21-bp insertion immediately 3' to the predicted FMO6 intron 8 splice acceptor site, diminishing the efficiency of this site.

L29 ANSWER 6 OF 73 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:893460 HCAPLUS
DOCUMENT NUMBER: 136:336165
TITLE: Modulation of survival motor neuron pre-mRNA **splicing** by inhibition of **alternative**

AUTHOR(S): Lim, Sharlene R.; Hertel, Klemens J.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA, 92697-4025, USA

SOURCE: Journal of Biological Chemistry (2001), 276(48), 45476-45483
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Spinal muscular atrophy is caused by the loss of functional survival motor neuron (SMN1) alleles. A translationally silent nucleotide transition in the duplicated copy of the gene (SMN2) leads to **exon** 7 **skipping** and expression of a nonfunctional gene product. It has been suggested that differential SMN2 splicing is caused by the disruption of an exonic splicing enhancer. Here we show that the single nucleotide

difference reduces the intrinsic strength of the 3' splice site of exon 7 2-fold, whereas the strength of the 5' splice site of the exon 7 is not affected. Thus, a decrease in splice site strength is magnified in the context of competing exons. These data suggest that lower levels of exon 7 definition not only reduce intron 6 removal but, more importantly, increase the efficiency of the competing **exon** 7 **skipping** pathway. Antisense oligonucleotides were tested to modulate exon 7 inclusion, which contains the authentic translation stop codon. Oligonucleotides directed toward the 3' splice site of exon 8 were shown to alter SMN2 splicing in favor of exon 7 inclusion. These results suggest that antisense oligonucleotides could be used as a **therapeutic** strategy to counteract the progression of SMA.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 7 OF 73 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001229125 MEDLINE
DOCUMENT NUMBER: 21181834 PubMed ID: 11285240
TITLE: Nuclear factor TDP-43 and **SR** proteins promote in vitro and in vivo **CFTR exon** 9 **skipping**.
AUTHOR: Buratti E; Dork T; Zuccato E; Pagani F; Romano M; Baralle F E
CORPORATE SOURCE: International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34012 Trieste, Italy.
SOURCE: EMBO JOURNAL, (2001 Apr 2) 20 (7) 1774-84.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010611
Last Updated on STN: 20010611
Entered Medline: 20010607

AB **Alternative splicing** of human **cystic fibrosis** transmembrane conductance regulator (**CFTR**) exon 9 is regulated by a combination of cis-acting elements distributed through the exon and both flanking introns (IVS8 and IVS9). Several studies have identified in the IVS8 intron 3' splice site a regulatory element that is composed of a polymorphic (TG)m(T)n repeated sequence. At present, no [illegible] have identified TDP-43, a nuclear protein not previously described to bind RNA, as the factor binding specifically to the (TG)m sequence. Transient TDP-43 overexpression in Hep3B cells results in an increase in **exon** 9 **skipping**. This effect is more pronounced with concomitant overexpression of **SR** proteins. Antisense inhibition of endogenous TDP-43 expression results in increased inclusion of exon 9, providing a new **therapeutic** target to correct **aberrant splicing** of exon 9 in CF patients. The clinical and biological relevance of this finding in vivo is demonstrated by our characterization of a CF patient carrying a TG10T9(DeltaF508)/TG13T3(wt) genotype leading to a disease-causing high proportion of **exon** 9 **skipping**
.

L29 ANSWER 8 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2001:123687 SCISEARCH
THE GENUINE ARTICLE: 396TC
TITLE: The CELF family of RNA binding proteins is implicated in

cell-specific and developmentally regulated **alternative splicing**

AUTHOR: Ladd A N; Charlet-B N; Cooper T A (Reprint)
CORPORATE SOURCE: Baylor Coll Med, Dept Pathol, Houston, TX 77030 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (FEB 2001) Vol. 21, No. 4, pp. 1285-1296.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0270-7306.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 68

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Alternative splicing** of cardiac troponin T (cTNT) exon 5 undergoes a developmentally regulated snitch such that exon inclusion predominates in embryonic, but not adult, striated muscle. We previously described four muscle-specific splicing enhancers (MSEs) within introns flanking exon 5 in chicken cTNT that are both necessary and sufficient for exon inclusion in embryonic muscle. We also demonstrated that CUG-binding protein (CUG-BP) binds a conserved CUG motif within a human cTNT MSE and positively regulates MSE-dependent exon inclusion. Here we report that CUG-BP is one of a novel family of developmentally regulated RNA binding proteins that includes embryonically lethal abnormal vision-type RNA binding protein 3 (ETR-3). This family, which we call CELF proteins for CUG-BP- and ETR-3-like factors, specifically bound MSE-containing RNAs in vitro and activated MSE-dependent exon inclusion of cTNT minigenes in vivo. The expression of two CELF proteins Is highly restricted to brain. CUG-BP, ETR-3, and CELF4 are more broadly expressed, and expression is developmentally regulated in striated muscle and brain. Changes in the level of expression and isoforms of ETR-3 in two different developmental systems correlated with regulated changes in cTNT splicing. A switch from cTNT **exon skipping** to inclusion tightly correlated with induction of ETR-3 protein expression during differentiation of C2C12 myoblasts. During heart development, the switch in cTNT splicing correlated with a transition in ETR-3 protein isoforms, We propose that ETR-3 is a major regulator of cTNT **alternative splicing** and that the CELF family plays an important regulatory role in cell-specific **alternative splicing** during **normal** development and disease.

L29 ANSWER 9 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:194795 HCAPLUS
DOCUMENT NUMBER: 137:183797
TITLE: Qualitative and quantitative analysis of mRNA associated with four putative splicing mutations (621+3A.fwdarw.G, 2751+2T.fwdarw.A, 296+1G.fwdarw.C, 1717-9T.fwdarw.C-D565G) and one nonsense mutation (E822X) in the **CFTR** gene
AUTHOR(S): Tzetis, Maria; Efthymiadou, Alexandra; Doudounakis, Stavros; Kanavakis, Emmanuel
CORPORATE SOURCE: Department of Medical Genetics, "Aghia Sophia" Children's Hospital, Athens University, Athens, 11527, Greece
SOURCE: Human Genetics (2001), 109(6), 592-601
CODEN: HUGEDQ; ISSN: 0340-6717
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal

Search completed by David Schreiber 308-4292

LANGUAGE: English

AB The effects of four splicing mutations and one nonsense mutation on **cystic fibrosis** transmembrane conductance regulator (**CFTR**) gene expression were investigated by reverse transcription-polymerase chain reaction anal. of mRNA extd. from nasal epithelial cells harvested from patients harboring the mutations. We studied four subjects with 621+3A.fwdarw.G, two with 2751+2T.fwdarw.A, one with 296+1G.fwdarw.C, two with 1717-9T.fwdarw.C-D565G and seven with E822X, and compared the results with **CFTR** mRNA from **normal** subjects. Our results showed that mutations 621+3A.fwdarw.G, 2751+2T.fwdarw.A, and 296+1G.fwdarw.C, which disrupt the 5' splice donor sites of introns 4, 14a, and 2, resp., and 1717-9T.fwdarw.C-D565G, which possibly disrupts the exonic splicing enhancer sequences of exon 12 (owing to the missense mutation in cis), lead to the prodn. of aberrantly spliced mRNA in nasal epithelial cells. Three of the splicing mutations (621+3A.fwdarw.G, 2751+2T.fwdarw.A, and 296+1G.fwdarw.C) result in severe deficiency of **normal** **CFTR** mRNA and severe phenotype in the patients. This information is esp. useful for mutation 621+3A.fwdarw.G, which is found in other populations as well, and was initially reported as a polymorphism. The complex allele 1717-9T.fwdarw.C-D565G results in **aberrant** **splicing** of **CFTR** mRNA with prodn. of transcripts lacking exon 12 (major product), with minor amts. of transcripts revealing joint **exon** 11 and 12 **skipping**. Nonsense mutation E822X results in a severe redn. in mRNA levels to about 6% of wild type. Patients with the mutation have a severe clin. phenotype, with both the pancreatic and the pulmonary function affected.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 10 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 2001:443651 SCISEARCH

THE GENUINE ARTICLE: 436GF

TITLE: Identification of an alternative transcript of ABCA1 gene in different human cell types

AUTHOR: Bellincampi L; Simone M L; Motti C; Cortese C; Bernardini S; Bertolini S; Calandra S (Reprint)

CORPORATE SOURCE: Univ Modena & Reggio Emilia, Dipartimento Sci Biomed, Via Campi 287, I-41100 Modena, Italy (Reprint); Univ Modena & Reggio Emilia, Dipartimento Sci Biomed, I-41100 Modena, Italy; Univ Rome Tor Vergata, Dept Med Interna, Rome, Teramo, Italy; Univ Genoa, Dipartimento Med Interna, Genoa, Italy

COUNTRY OF AUTHOR: Italy

SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (11 MAY 2001) Vol. 283, No. 3, pp. 590-597.
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.
ISSN: 0006-291X.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have observed two ABCA1 gene transcripts in human skin fibroblasts, The RT-PCR amplification of the exon 3-exon 8 region generated a **normal** fragment (740 bp) and an abnormal fragment (600 bp) in a ratio ranging from 3:1 to 8/9:1. These two transcripts were present in other cells such as leukemia T-cells, endothelial and smooth muscle cells

as well human hepatoma cells (HepG2). Restriction enzyme analysis and sequencing indicated that in the abnormal fragment exon 3 was followed by **exon** 5. The complete **skipping** of **exon** 4 leads to a premature stop and a predicted translation product of 74 amino acids. The ratio between the **normal** and alternative transcript is not affected by variation in ABCA1 gene expression induced by incubating cells in serum-free medium and in the presence of cholesterol. It is possible that this **alternative splicing** represents as mechanism that regulates the ABCA1 content in tissues. (C) 2001 Academic Press.

L29 ANSWER 11 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:153060 BIOSIS
DOCUMENT NUMBER: PREV200200153060
TITLE: p210BCR/ABL-induced alteration of mRNA splicing as a potential mechanism of CML pathogenesis.
AUTHOR(S): Salesse, Stephanie (1); Dylla, Scott J. (1); Verfaillie, Catherine M. (1)
CORPORATE SOURCE: (1) Stem Cell Institute, University of Minnesota Cancer Center, Minneapolis, MN USA
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 144a. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB Chronic Myelogenous Leukemia (CML) is a malignancy of the human hematopoietic stem cell (HSC) characterized by presence of the BCR/ABL gene and its product, p210BCR/ABL. The exact mechanism(s) underlying p210BCR/ABL-mediated transformation is not totally understood. Pre-mRNA processing is an important step in the transition from DNA to protein, wherein exons likely require different groups of trans-acting factors and cis-acting elements for their proper union. Although **alternative splicing** of mRNA is a critical determinant of a cell's protein repertoire, it has never been implicated in CML pathogenesis. Subtractive hybridization techniques comparing MSCV-IRES-GFP and MSCV-p210BCR/ABL-IRES-GFP transduced cord blood (CB) CD34+ cells identified multiple genes involved in pre-mRNA splicing in BCR/ABL-positive cells, including the **SR**-protein kinase 1 (**SRPK1**) and RNA Helicase II/Gu. Northern blot, quantitative real-time PCR, and Western blot analyses have confirmed increased expression of these genes and their products in primary CML versus **normal** CD34+ cells and p210BCR/ABL versus GFP-transduced CB CD34+ cells. Moreover, the phosphorylation of **SRPK1** substrates, such as **SR** proteins, which are critical components of the spliceosome complex, is increased in p210BCR/ABL-containing cells. Intriguingly, previous experiments in our lab investigating the abnormal adhesion and migration of BCR/ABL-positive cells characterized **alternatively splicing** of the non-receptor tyrosine kinase gene, PYK2, whose product is intimately involved in beta1-integrin signaling. In p210BCR/ABL-positive cells, the ratio of full-length Pyk2 versus the PYK2 isoform **normally** predominant in hematopoietic cells, Pyk2H, is increased. **Alternative splicing** of PYK2 is directly influenced by p210BCR/ABL as indicated by the significantly higher ratio of Pyk2/Pyk2H in primary CML versus **normal** CD34+ cells and p210BCR/ABL versus GFP-transduced CB CD34+ cells. Moreover, **treatment** of primary CML CD34+ cells or p210BCR/ABL-transduced CB CD34+ cells with the Abl-specific tyrosine kinase inhibitor, STI571, **normalized** the

Pyk2/Pyk2H ratio. This STI571-induced reversion to **normal** PYK2 gene splicing correlated with decreased expression of the splicing proteins **SRPK1** and RNA Helicase II/Gu, and reduced phosphorylation of the **SR** proteins. Reversible phosphorylation of **SR** proteins is critical for spliceosome assembly and subsequent splicing, and **SRPK1** overexpression inhibits pre-mRNA splicing (Genes Dev 1996 10:1569). Therefore, experiments are in progress in which **SRPK1** is overexpressed in **normal** CB CD34+ cells, by MSCV-based retroviral transduction, to determine whether the BCR/ABL-induced alteration of PYK2 mRNA splicing is mediated solely by the elevation of **SRPK1** expression/activity, and whether these changes in **splicing** contribute to the **abnormal** adhesion and migration characteristics observed in CML.

L29 ANSWER 12 OF 73 MEDLINE
ACCESSION NUMBER: 2000388548 MEDLINE
DOCUMENT NUMBER: 20267225 PubMed ID: 10809228
TITLE: An estrogen receptor-alpha splicing variant mediates both positive and negative effects on gene transcription.
AUTHOR: Bollig A; Miksicek R J
CORPORATE SOURCE: Michigan State University, Department of Physiology, East Lansing 48824-1101, USA.
SOURCE: MOLECULAR ENDOCRINOLOGY, (2000 May) 14 (5) 634-49. Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000810

AB Analysis of mRNA prepared from a variety of estrogen-responsive cell lines, breast tumor specimens, and **normal** breast tissue have established that estrogen receptor-alpha (ER alpha) mRNA is typically expressed as a mixture of transcripts. Using PCR amplification, this heterogeneity has been shown to result largely from an imprecise pattern of mRNA splicing that gives rise to a family of correctly processed and **exon-skipped** ER alpha transcripts. We have reconstructed ER alpha cDNAs representing the single **exon-skipped** variants ERdeltaE2 through ERdeltaE7 to enable their functional characterization in a well defined cell transfection system. All six of the ER alpha splicing variants support the efficient expression of stable proteins in Cos7 cells, and each shows a characteristic pattern of subcellular distribution. Each of the variants displays a dramatic reduction in DNA-binding activity with a consensus estrogen response element (ERE) in an in vitro gel mobility shift assay. While this DNA-binding defect appears to be complete for ERdeltaE2, ERdeltaE3, ERdeltaE4, and ERdeltaE6, weak DNA binding is observed for ERdeltaE5 and ERdeltaE7. Scatchard analysis of hormone binding demonstrates that among the variants, only ERdeltaE3 binds 17beta-estradiol (E2) and does so with an affinity similar to wild-type ER alpha (wt ER alpha). Individual variants cotransfected with the pERE-TK-CAT reporter plasmid [a consensus ERE-driven chloramphenicol acetyltransferase (CAT) reporter gene that is highly responsive to E2-liganded wt ER alpha] were ineffective at inducing CAT expression in ER-negative HeLa cells. Only ERdeltaE5 showed indications of positive transcriptional activity on the pERE-TK-CAT reporter, but this activity was limited to approximately 5% of the activity of wt ER alpha. When variants were expressed simultaneously with

wt ER alpha, ERdeltaE3 and ERdeltaE5 were observed to have a dominant negative effect on wt ER alpha transcriptional activity. Like the wild-type receptor, both ERdeltaE3 and ERdeltaE5 interact with steroid receptor coactivator-1e (**SRC**-1e) in vitro; however, only ERdeltaE3 retained the ability to dimerize with wt ER alpha. Transcription from a region of the ovalbumin promoter, which contains an ERE half-site and an AP-1 motif, is positively regulated by liganded wt ER alpha and ERdeltaE3 in phorbol ester-**treated**, transiently transfected HeLa cells. In both cases, this activity was enhanced by cotransfected cJun. These observations suggest that selected ER alpha splicing variants are likely to exert important transcriptional effects, especially on genes that are regulated by nonconsensus EREs and subject to complex hormonal control.

L29 ANSWER 13 OF 73 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001023142 MEDLINE
DOCUMENT NUMBER: 20455571 PubMed ID: 10998326
TITLE: Identification and elimination of an **aberrant splice** product from cDNAs encoding the human adenovirus type 5 **E4orf6** protein.
AUTHOR: Querido E; Chu-Pham-Dang H; Branton P E
CORPORATE SOURCE: Department of Biochemistry, McGill University, Montreal, Quebec, H3G 1Y6, Canada.
SOURCE: VIROLOGY, (2000 Sep 30) 275 (2) 263-6.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001107

AB Growing awareness of the central role of the **E4orf6** protein in regulating the infectious cycle of human adenoviruses has led to greatly intensified efforts to define its functions and mechanisms of action. Many workers employ cDNAs to generate plasmid or viral vectors to express **E4orf6** in the absence of other viral products. In addition to the **normal** 34-kDa product, such vectors consistently produce a polypeptide of about 8 kDa. In the present report we show that this protein is produced by an aberrant mRNA utilizing the 5' splice donor site used normally by the virus to produce the E4orf6/7 product, which shares 58 residues with **E4orf6**. This amino terminal coding sequence is linked to a 3' sequence via a novel splice acceptor site in an alternative reading frame of the **E4orf6** cDNA. The 5' donor site was altered by PCR-directed mutagenesis to yield a construct that produces high levels of **E4orf6** in the absence of the 8-kDa polypeptide. This construct should eliminate some of the problems encountered previously using the wild-type **E4orf6** coding sequence.

L29 ANSWER 14 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
ACCESSION NUMBER: 2001:489135 BIOSIS
DOCUMENT NUMBER: PREV200100489135
TITLE: Repression of **aberrant splicing** in human beta-globin pre-mRNA with HbE mutation by antisense oligoribonucleotide or splicing factor SF2/ASF.

Search completed by David Schreiber 308-4292

Kam 09/871,809

AUTHOR(S): Shirohzu, Hisao; Yamaza, Haruyoshi; Fukumaki, Yasuyuki (1)
CORPORATE SOURCE: (1) Division of Disease Genes, Institute of Genetic Information, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582: yfukumak@gen.kyushu-u.ac.jp Japan
SOURCE: International Journal of Hematology, (July, 2000) Vol. 72, No. 1, pp. 28-33. print.
ISSN: 0925-5710.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Hemoglobin (Hb) E is the most common Hb variant among Southeast Asian populations. The mutation in codon 26 (GAG to AAG) of the beta-globin gene (betaE) induces **alternative splicing**, resulting in the production of **normally** and aberrantly spliced beta-globin mRNA. Compound heterozygosity for beta-thalassemia and HbE, beta-thalassemia/HbE disease, could lead to a severe thalassemia phenotype. Repression of **aberrant splicing** from the betaE mutation could ameliorate the severity in such patients. We showed that the **aberrant splicing** was partially repressed in cells **treated** with antisense oligoribonucleotide targeted to the **aberrant** 5' **splice** site. The maximum effect of the antisense oligoribonucleotide was observed at a concentration of 0.4 mumol/L, 36 hours after the **treatment** in our experiment. We also analyzed the effect of the transient and stable expression of SF2/ASF on **aberrant splicing** in cells expressing the betaE-globin gene. Partial repression of the **aberrant splicing** was also observed in both expression systems. Our results imply that antisense oligoribonucleotide **treatment** and SF2/ASF expression are possible **therapeutic** applications for beta-thalassemia/HbE disease.

L29 ANSWER 15 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:327679 HCAPLUS
TITLE: Modification of splicing pathways by antisense oligonucleotides.
AUTHOR(S): Kole, Ryszard
CORPORATE SOURCE: Lineberger Comprehensive Cancer Center and Department of Pharmacology, University of North Carolina, Chapel Hill, NC, 27599, USA
SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), CARB-003. American Chemical Society: Washington, D. C.
CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

AB Blocking of **aberrant** or **alternative splice** sites with antisense oligonucleotides led to restoration or modification of expression of several clin. relevant genes. In cells recapitulating .beta.-thalassemia or **cystic fibrosis** caused by splicing mutations the antisense oligonucleotides not only inhibited **aberrant splicing** but also restored correct splicing in a dose dependent and sequence specific fashion, generating correct human .beta.-globin and **CFTR** mRNAs and polypeptides. Similarly, antisense oligonucleotides restored a reading frame in the mutated murine dystrophin gene in cultured myotubes from mdx mice. Thus, the antisense oligonucleotide **treatments** provide potential alternatives to gene replacement **therapies**. Recent results show that modification of splicing by antisense oligonucleotides is also useful as potential anti-cancer **therapy**. The effects of oligonucleotide

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chem. in the modification of splicing will be discussed for these applications.

L29 ANSWER 16 OF 73 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1999445537 MEDLINE
DOCUMENT NUMBER: 99445537 PubMed ID: 10514463
TITLE: The relative strengths of **SR** protein-mediated associations of alternative and constitutive exons can influence **alternative splicing.**
AUTHOR: Stark J M; Cooper T A; Roth M B
CORPORATE SOURCE: Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, USA.
CONTRACT NUMBER: GM48435 (NIGMS)
HL45565 (NHLBI)
T32 GM07270 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Oct 15) 274 (42) 29838-42.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991119

AB We have characterized the functional role of **SR** protein-mediated exon/exon associations in the **alternative splicing** of exon 5 of chicken cardiac troponin T (cTnT). We have previously shown that **SR** proteins can promote the association of the alternative exon 5 with the flanking constitutive exon 6 of this pre-mRNA. In this study, we have shown that when exons 2, 3, and 4 of the cTnT pre-mRNA are spliced together, the composite exon 2/3/4 contains an additional **SR** protein binding site. Furthermore, we have found that **SR** proteins can also promote interactions between the pairs of exons 2/3/4-5 and 2/3/4-6. We then asked whether the **SR** protein binding sites in these exons play a role in cTnT **alternative splicing** in vivo. We found that the **SR** protein binding sites in exons 2/3/4 and 6 promote **exon** 5 **skipping,** and it has previously been shown that the **SR** protein binding site in exon 5 promotes exon 5 inclusion. Consistent with these results, we find that the **SR** protein-mediated association of exon 2/3/4 with 6 is preferred over associations involving exon 5, in that exons 2/3/4 and 6 are more efficient than exon 5 in competing an **SR** protein-mediated exon/exon association. We suggest that the relative strengths of **SR** protein-mediated associations of alternative and constitutive exons play a role in determining **alternative splicing** patterns.

L29 ANSWER 17 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2000:31676 SCISEARCH
THE GENUINE ARTICLE: 270GB
TITLE: Differential expression of the splicing regulatory factor genes during two-step chemical transformation in a BALB/3T3-derived cell line, MT-5
AUTHOR: Maeda T (Reprint); Hiranuma H; Jikko A
CORPORATE SOURCE: OSAKA UNIV, SCH DENT, DEPT RADIOL & RADIAT ONCOL, 1-8 YAMADAOKA, OSAKA 5650871, JAPAN (Reprint)
COUNTRY OF AUTHOR: JAPAN

Kam 09/871,809

SOURCE: CARCINOGENESIS, (DEC 1999) Vol. 20, No. 12, pp. 2341-2344.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0143-3334.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 24

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Although the **alternative splicing** of various genes is a common event in human tumors, the mechanisms behind it have not been characterized. We hypothesized that the expression of splicing regulatory factors would be changed during cellular transformation. Gene expression of three **splicing** regulatory factors, **alternative splicing** factor/**splicing** factor 2 (ASF/SF2), heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2) and the 65 kDa subunit of U2 small nuclear ribonucleoprotein particles auxiliary factor (U2AF(65)), were examined by northern blotting in a two-step chemical transformation model, This in vitro model is composed of BALB/3T3 cells and a BALB/3T3-derived N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-initiated cell line (MT-5), MT-5 cells can be transformed on exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA), ASF/SF2 mRNA levels were decreased 2-fold in both MNNG-initiated cells and TPA-induced transformed cells compared with the **normal** parental cells, whereas hnRNP A2 mRNA expression did not significantly change between these three types of cells. U2AF(65) mRNA levels were markedly increased (similar to 4.7-fold) associated with progression of cellular transformation. Moreover, RT-PCR analysis showed that distinct forms of ASF/SF2 mRNA were present in the MNNG-initiated cells and TPA-induced transformed cells but not in the parental cells, These findings indicate that ASF/SF2 or U2AF(65) gene expression is altered during in vitro two-step chemical transformation. The data suggest that the differential expression of splicing regulatory factors is one cause of **aberrant** expression of **alternatively spliced** mRNAs encoded by various genes in tumor cells.

L29 ANSWER 18 OF 73 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000025742 MEDLINE
DOCUMENT NUMBER: 20025742 PubMed ID: 10556281
TITLE: Functional analysis of cis-acting elements regulating the **alternative splicing** of human **CFTR** exon 9.
AUTHOR: Niksic M; Romano M; Buratti E; Pagani F; Baralle F E
CORPORATE SOURCE: International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34012, Trieste, Italy.
SOURCE: HUMAN MOLECULAR GENETICS, (1999 Dec) 8 (13) 2339-49.
Journal code: 9208958. ISSN: 0964-6906.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF176095; GENBANK-AF176096
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000204
Last Updated on STN: 20000204
Entered Medline: 20000124

AB The rate of exon 9 exclusion from the **cystic fibrosis** transmembrane conductance regulator (**CFTR**) mRNA is associated

Search completed by David Schreiber 308-4292

with monosymptomatic forms of **cystic fibrosis**. Exon 9 **alternative splicing** is modulated by a polymorphic polythymidine tract within its 3' splice site. We have generated a minigene carrying human **CFTR** exon 9 with its flanking intronic sequences and set up an in vivo model to study the cis-acting DNA elements which modulate its splicing. Transfections into human cell lines showed that T5, but not T9 or T7 alleles, significantly increases the **alternative splicing** of exon 9. Moreover, we found that another polymorphic locus juxtaposed upstream of the T tract, and constituted by (TG)(n)repeats, can further modulate **exon** 9 **skipping** but only when activated by the T5 allele. Then, we extended our studies to the mouse **CFTR** exon 9 which does not show **alternative splicing**. Comparison of human and mouse introns 8 and 9 revealed a low homology between the two sequences and the absence of the human polymorphic loci within the mouse intron 3' splice site. We have tested a series of constructs where the whole human exon 9 with its flanking intronic sequences was replaced partially or completely by the murine counterpart. The transfections of these constructs in human and murine cell lines reveal that also sequences of the downstream intron 9 affect exon 9 definition and co-modulate, with the UG/U 3' splice site sequences, the extent of **exon** 9 **skipping** in **CFTR** mRNA.

L29 ANSWER 19 OF 73 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1999219872 MEDLINE
DOCUMENT NUMBER: 99219872 PubMed ID: 10202157
TITLE: Modulation of **exon skipping** by high-affinity hnRNP **A1**-binding sites and by intron elements that repress splice site utilization.
AUTHOR: Blanchette M; Chabot B
CORPORATE SOURCE: Departement de Microbiologie et d'Infectiologie, Faculte de Medecine, Universite de Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4.
SOURCE: EMBO JOURNAL, (1999 Apr 1) 18 (7) 1939-52.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 20021219
Entered Medline: 19990601

AB The RNA-binding protein hnRNP **A1** is a splicing regulator produced by exclusion of alternative exon 7B from the **A1** pre-mRNA. Each intron flanking exon 7B contains a high-affinity **A1**-binding site. The **A1**-binding elements promote **exon skipping** in vivo, activate distal 5' splice site selection in vitro and improve the responsiveness of pre-mRNAs to increases in the concentration of **A1**. Whereas the glycine-rich C-terminal domain of **A1** is not required for binding, it is essential to activate the distal 5' splice site. Because **A1** complexes can interact simultaneously with two **A1**-binding sites, we propose that an interaction between bound **A1** proteins facilitates the pairing of distant splice sites. Based on the distribution of putative **A1**-binding sites in various pre-mRNAs, an **A1**-mediated change in pre-mRNA conformation may help define the borders of mammalian introns. We also identify an intron element which represses the 3' splice site of exon 7B. The activity of this

element is mediated by a factor distinct from **A1**. Our results suggest that **exon** 7B **skipping** results from the concerted action of several intron elements that modulate splice site recognition and pairing.

L29 ANSWER 20 OF 73 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1999190506 MEDLINE
DOCUMENT NUMBER: 99190506 PubMed ID: 10092085
TITLE: SF2 and **SRp55** regulation of CD45 **exon** 4 **skipping** during T cell activation.
AUTHOR: Lemaire R; Winne A; Sarkissian M; Lafyatis R
CORPORATE SOURCE: Boston University School of Medicine, The Arthritis Center, MA 02118, USA.
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (1999 Mar) 29 (3) 823-37. Journal code: 1273201. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 19990504
Entered Medline: 19990421

AB CD45 is an **alternatively spliced** membrane phosphatase required for T cell activation. Exons 4, 5 and 6 can be included or skipped from spliced mRNA resulting in several protein isoforms that include or exclude epitopes referred to as RA, RB or RC, respectively. T cells reciprocally express CD45RA or CD45RO (lacking all three exons), corresponding to naive versus memory T cells. Overexpression of the **alternative splicing** regulators, SF2 or SWAP, induces **skipping** of CD45 **exon** 4 in transfected COS cells. We show here that the **arginine/serine-rich** domain of SWAP and the RNA recognition motifs of SF2 are required for **skipping** of CD45 **exon** 4. Unlike SWAP, SF2 specifically regulated **alternative splicing** of CD45 exon 4, having no effect on a non-regulated exon of CD45 (exon 9). Like SF2 and SWAP, the **SR** proteins SC35, **SRp40** and **SRp75**, but not **SRp55** also induced CD45 **exon** 4 **skipping**. In contrast, antisense inhibition of **SRp55** induced **exon** 4 **skipping**. SF2 and **SRp55** proteins were not detectable or expressed at a very low level in freshly isolated CD45RA+ and CD45RO+ T cells. Activation of CD45RA+ T cells shifted CD45 expression from CD45RA to CD45RO, and induced a large increase in expression of both SF2 and **SRp55**. Thus, SF2 at least in part determines splicing of CD45 exon 4 during T cell activation. **SRp55**, **SR** protein phosphorylation, or other splicing factors likely regulate CD45 splicing in CD45RO+ memory T cells. The different **SR** proteins expressed by memory and activated T cells emphasize the different phenotypes of these cell types that both express CD45RO.

L29 ANSWER 21 OF 73 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 2000051033 MEDLINE
DOCUMENT NUMBER: 20051033 PubMed ID: 10583508
TITLE: Cloning and characterization of two neural-salient **serine/arginine-rich** (NSSR) proteins involved in the regulation of **alternative splicing** in neurones.
AUTHOR: Komatsu M; Kominami E; Arahata K; Tsukahara T

Search completed by David Schreiber 308-4292

CORPORATE SOURCE: Department of Neuromuscular Research, National Institute of Neuroscience, NCNP, Ogawahigashi 4-1-1, Kodaira, Tokyo 187-8502, Japan; Department of Biochemistry, Juntendo University School of Medicine, Tokyo, Japan.
SOURCE: GENES TO CELLS, (1999 Oct) 4 (10) 593-606.
Journal code: 9607379. ISSN: 1356-9597.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB015894; GENBANK-AB015895
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000113

AB BACKGROUND: In neurones, **alternative splicing** regulates the functions of many gene products. However, the molecular basis of neural-specific splicing, and how splicing regulation is modulated in different neurones remains to be determined. RESULTS: We cloned two new **SR** proteins, Neural-salient **SR** proteins (NSSR) 1 and 2, which are present at higher levels in brain and testis. During the differentiation, NSSR 1 is detected only in the neuronal stage. Both the purified recombinant NSSR 1 and 2 proteins enhance the in vitro splicing activity of nuclear extract. Moreover, recombinant NSSR 1 protein enhances the assembly of ribonucleoprotein complexes with S100 fraction. Over-expression of NSSR 2 prevents the inclusion of either the Flip or Flop exons in the splicing of the GluR-B gene, resulting in an increase in the abnormal **exon-skipping** product. In contrast, transient transfection with NSSR 1 promotes the inclusion of the Flip exon so that the **abnormal** product is **spliced** to the mature spliced form. This suppression of **exon skipping** by NSSR 1 is observed even with co-transfection of NSSR 2. CONCLUSIONS: NSSR 1 and 2 were cloned from mouse cDNA libraries. Results indicate that NSSR 1 may play a crucial role in the regulation of **alternative splicing** in neurones.

L29 ANSWER 22 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:554255 HCAPLUS
DOCUMENT NUMBER: 132:91514
TITLE: Inactivation patterns of the p16 (INK4a) gene in oral squamous cell carcinoma cell lines
AUTHOR(S): Akapuma, D.; Uzawa, N.; Yoshida, M. A.; Negishi, A.; Amagasa, T.; Ikeuchi, T.
CORPORATE SOURCE: Department of Molecular Cytogenetics, Medical Research Institute, First Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, 113-0034, Japan
SOURCE: Oral Oncology (1999), 35(5), 476-483
CODEN: EJCCER; ISSN: 1368-8375
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To det. whether inactivation of the p16 gene mapped to the chromosome 9p21 region is assocd. with the development of oral squamous-cell carcinoma (SCC), the authors investigated the mutational states of two forms of alternative transcripts (.alpha. and .beta.) from the p16 gene in 14 oral SCC cell lines by RT-PCR, PCR, direct sequencing, and methylation analyses. Alterations of the .alpha. transcript were detected in all of the cell lines examd.: homozygous deletions in three lines; subtle

mutations in exons 1.alpha. or 2 in four lines; **skipping** of **exon** 2 in two lines; hypermethylation of the 5' CpG island of the p16 gene in four lines; and an unknown mechanism in one line. Abnormalities of the .beta. transcript were obsd. in seven of the 14 cell lines. Nonetheless, the mutations that essentially affect the function of the encoded protein were found only in five cell lines, including three lines with homozygous deletion. There was no cell line having only .beta. transcript alterations. Thus, alteration of the .alpha. transcript of the p16 gene was a highly frequent event in oral SCC. Since this type of alteration resulted in gene inactivation through multiple pathways, it may play a major role in the process of oral SCC development.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 23 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:206648 SCISEARCH
THE GENUINE ARTICLE: 173YK
TITLE: Sensitivity of splice sites to antisense oligonucleotides in vivo
AUTHOR: Sierakowska H; Sambade M J; Schumperli D; Kole R (Reprint)
CORPORATE SOURCE: UNIV N CAROLINA, LINEBERGER COMPREHENS CANC CTR, CB 7295, CHAPEL HILL, NC 27599 (Reprint); UNIV N CAROLINA, LINEBERGER COMPREHENS CANC CTR, CHAPEL HILL, NC 27599; UNIV N CAROLINA, DEPT PHARMACOL, CHAPEL HILL, NC 27599; UNIV BERN, INST ZOOL, ABT ENTWICKLUNGSBIOL, CH-3012 BERN, SWITZERLAND
COUNTRY OF AUTHOR: USA; SWITZERLAND
SOURCE: RNA-A PUBLICATION OF THE RNA SOCIETY, (MAR 1999) Vol. 5, No. 3, pp. 369-377.
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
ISSN: 1355-8382.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A series of HeLa cell lines which stably express beta-globin pre-mRNAs carrying point mutations at nt 654, 705, or 745 of intron 2 has been developed, The mutations generate **aberrant** 5' **splice** sites and activate a common 3' cryptic splice site upstream leading to aberrantly spliced beta-globin mRNA. Antisense oligonucleotides, which in vivo blocked **aberrant splice** sites and restored correct splicing of the pre-mRNA, revealed major differences in the sensitivity of these sites to antisense probes. Although the targeted pre-mRNAs differed only by single point mutations, the effective concentrations of the oligonucleotides required for correction of splicing varied up to 750-fold. The differences among the **aberrant** 5' **splice** sites affected sensitivity of both the 5' and 3' splice sites; in particular, sensitivity of both splice sites was severely reduced by modification of the **aberrant** 5' **splice** sites to the consensus sequence. These results suggest large differences in splicing of very similar pre-mRNAs in vivo, They also indicate that antisense oligonucleotides may provide useful tools for studying the interactions of splicing machinery with pre-mRNA.

L29 ANSWER 24 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:834075 SCISEARCH
THE GENUINE ARTICLE: 249ND

Search completed by David Schreiber 308-4292

Kam 09/871,809

TITLE: **Alternatively spliced** mRNA molecules of the thrombospondin receptor (CD36) in human PBMC
AUTHOR: Kern P; Kolowos W; Hagenhofer M; Frank C; Kalden J R; Herrmann M (Reprint)
CORPORATE SOURCE: UNIV ERLANGEN NURNBERG, INST CLIN IMMUNOL, KRANKENHAUSSTR 12, D-91054 ERLANGEN, GERMANY (Reprint); UNIV ERLANGEN NURNBERG, INST CLIN IMMUNOL & RHEUMATOL, DEPT INTERNAL MED 3, D-91054 ERLANGEN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: EUROPEAN JOURNAL OF IMMUNOGENETICS, (OCT 1999) Vol. 26, No. 5, pp. 337-342.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 0960-7420.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We employed reverse transcription polymerase chain reaction (RT-PCR) to detect **alternatively spliced** CD36 mRNA in human peripheral blood mononuclear cells (PBMC). Sequencing of cloned cDNA revealed **alternatively spliced** mRNA molecules in 13 out of 39 clones. We observed **exon skipping** of up to 10 out of 12 coding exons in eight alternative transcripts. Additionally, in five of the transcripts, **alternative splice** donor or acceptor sites were used during mRNA maturation. Considering the CD36 molecule serves many functions in coagulation, host defence, lipid metabolism, and scavenging, we speculate that the proteins encoded by the **alternatively spliced** mRNA molecules may be involved in regulation of both CD36 gene expression and function.

The human CD36 molecule is a 78-88 kDa glycoprotein belonging to the class B scavenger receptor (**SR**) gene family (Endemann et al., 1993). In humans the gene has been localized to chromosome 7 (7q11.2 band) (Fernandez-Ruiz et al., 1993). The protein has two hydrophobic amino acid domains. located at both the carboxy and the amino terminals. Soluble carboxy-terminal truncation mutants binding to thrombospondin showed evidence for a single transmembrane region (carboxy-terminal) (Pearce et al., 1994). However; controversial data (Tao et al., 1996) suggest the amino terminal hydrophobic region is associated with the outer surface of the cytoplasmic membrane. Six carboxy-terminal amino acids are reported to be located in the cytoplasm (Vega et al., 1991). If there were a second amino terminal transmembrane region, another six amino acids would be located in the cytoplasm. The single large extracellular domain contains most of the potential N-glycosylation sites in its amino-terminal region, whereas the carboxy-terminal region is rich in conserved proline, glycine and cysteine residues (Greenwalt et al., 1992).

L29 ANSWER 25 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:116161 HCAPLUS
DOCUMENT NUMBER: 130:309835
TITLE: The same mutation affecting the splicing of WT1 gene is present on Frasier syndrome patients with or without Wilms' tumor
AUTHOR(S): Barbosa, Angela S.; Hadjiathanasiou, Charalambos G.; Theodoridis, Charalambos; Papathanasiou, Asteroula; Tar, Attila; Merksz, Miklos; Gyorvari, Borbala; Sultan, Charles; Dumas, Robert; Jaubert, Francis; Niaudet, Patrick; Moreira-Filho, Carlos A.; Cotinot,

Search completed by David Schreiber 308-4292

Corinne; Fellous, Marc
CORPORATE SOURCE: Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
SOURCE: Human Mutation (1999), 13(2), 146-153
CODEN: HUMUE3; ISSN: 1059-7794
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Denys-Drash and Frasier syndromes are rare human disorders that assoc. nephropathy with gonadal and genital abnormalities. In DDS there is a predisposition to Wilms' tumor. Heterozygous point mutations in the Wilms' tumor, type 1 gene (WT1), particularly those altering the zinc finger (ZF) encoding exons, have been reported in most DDS patients, while mutations in intron 9 of the same gene cause FS. This paper describes two cases of DDS, one FS and one patient with Wilm's tumor and intersex genitalia, in which mutations were searched by sequencing the exons 8 and 9 of WT1 gene. Patient 1 carried a missense (sic) point mutation in exon 8 (ZF2), converting a CGA-Arg codon to a TGA-stop codon. Patient 2 presented a single nucleotide deletion within exon 9 (ZF3) introducing a premature chain termination at codon 398. Patients 3 and 4 had a **C.fwdarw.T** transition at position +4 of the second **alternative splice** donor site of exon 9 (this mutation was detected in peripheral blood and in tumor derived DNA of patient 3). However, patient 3 had previously developed a Wilms' tumor. This is the first case of Wilms' tumor development in a phenotypically and genetically confirmed case of FS.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 26 OF 73 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 1999246057 MEDLINE
DOCUMENT NUMBER: 99246057 PubMed ID: 10231026
TITLE: Structural organization of the human Elk1 gene and its processed pseudogene Elk2.
AUTHOR: Yamauchi T; Toko M; Suga M; Hatakeyama T; Isobe M
CORPORATE SOURCE: Department of Materials and Biosystem Engineering, Faculty of Engineering, Toyama University, Toyama-city, Japan.
SOURCE: DNA RESEARCH, (1999 Feb 26) 6 (1) 21-7.
Journal code: 9423827. ISSN: 1340-2838.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB016193; GENBANK-AB016194; GENBANK-AB016195
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990730
Last Updated on STN: 19990730
Entered Medline: 19990720
AB In the ets gene family of transcription factors, ELK1 belongs to the subfamily of Ternary Complex Factors (TCFs) which bind to the Serum Response Element (**SRE**) in conjunction with a dimer of Serum Response Factors (**SRFs**). The primary structure of the human Elk1 gene was determined by genomic cloning. The gene structure of Elk1 spans 15.2 kb and consists of seven exons and six introns. The coding sequence resides on exons 3, 4, 5, 6 and 7. Sequencing of cDNA clones isolated from human hippocampus library revealed that the second **exon** was often **skipped** by an **alternative splicing** event. All introns commenced with nucleotides GT at the 5' boundary and ended with nucleotides AG at the 3' boundary, in agreement

with the proposed consensus sequence for intron spliced donor and acceptance sites. Sequence inspection of the 5'-flanking region revealed the absence of a 'TATA' box and the presence of putative cis-acting regulatory elements such as Sp1, GATA-1, CCAAT, and c-Myb. Moreover, the sequence analysis of Elk2 locus on 14q32.3 confirmed that Elk2 gene corresponds to a processed pseudogene of Elk1 which has been reported between alpha 1 gene (IGHA1) and pseudo gamma gene (IGHGP) of immunoglobulin heavy chain. Furthermore, the results of Southern analysis using DNAs from human-mouse hybrid cell lines carrying a part of 14q32 region revealed that there is another locus hybridizing to Elk1 cDNA on 14q32.2 --> qter region in addition to Elk2 locus between IGHA1 and IGHGP loci.

L29 ANSWER 27 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:326279 SCISEARCH
THE GENUINE ARTICLE: ZJ519
TITLE: Identification of intron and exon sequences involved in **alternative splicing** of insulin receptor pre-mRNA
AUTHOR: Kosaki A (Reprint); Nelson J; Webster N J G
CORPORATE SOURCE: VET ADM MED CTR, MED RES SERV, SAN DIEGO, CA 92161; UNIV CALIF SAN DIEGO, CTR CANC, LA JOLLA, CA 92093; UNIV CALIF SAN DIEGO, DEPT MED, DIV ENDOCRINOL & METAB, WHITTIER DIABET RES PROGRAM, LA JOLLA, CA 92093
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (24 APR 1998) Vol. 273, No. 17, pp. 10331-10337.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The insulin receptor exists as two isoforms, A and B, that result from **alternative splicing** of exon 11 in the primary transcript. We have shown previously that the **alternative splicing** is developmentally and hormonally regulated. Consequently, these studies were instigated to identify sequences within the primary RNA transcript that regulate the **alternative splicing**. Minigenes containing exons 10, 11, and 12 and the intervening introns were constructed and transfected into HepG2 cells, which contain both isoforms of the insulin receptor. The cells were able to splice the minigene transcript to give both A (- exon 11) and B-like (+ exon 11) RNAs. A series of internal deletions within intron 10 were tested for their ability to give A and B RNAs. Intron 10 contained two sequences that modulated exon 11 inclusion; a 48-nucleotide purine-rich sequence at the 5' end of intron 10 that functions as a splicing enhancer and causes an increase in exon 11 inclusion, and a 43-nucleotide sequence at the 3' end of intron 10 upstream of the branch point sequence that favors **skipping** of **exon** 11. Increasing the length of the polypyrimidine tract at the 3' end of intron 10 caused exon 11 to be spliced constitutively, indicating that a weak splice site is required for **alternative splicing**. Finally, point mutations, insertions, and deletions within exon 11 itself were able to regulate inclusion of the exon both positively and negatively.

L29 ANSWER 28 OF 73 MEDLINE DUPLICATE 12

Search completed by David Schreiber 308-4292

Kam 09/871,809

ACCESSION NUMBER: 1998356127 MEDLINE
DOCUMENT NUMBER: 98356127 PubMed ID: 9689050
TITLE: Regulation of Ich-1 pre-mRNA **alternative splicing** and apoptosis by mammalian splicing factors.
AUTHOR: Jiang Z H; Zhang W J; Rao Y; Wu J Y
CORPORATE SOURCE: Department of Pediatrics and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, USA.
CONTRACT NUMBER: RO1 GM53945 (NIGMS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Aug 4) 95 (16) 9155-60. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980917
Last Updated on STN: 20021218
Entered Medline: 19980908

AB The importance of **alternative splicing** in regulating apoptosis has been suggested by findings of functionally antagonistic proteins generated by **alternative splicing** of several genes involved in apoptosis. Among these, Ich-1 (also named as caspase-2) encodes a member of the caspase family of proteases. Two forms of Ich-1 are produced as a result of **alternative splicing**: Ich-1L, which causes apoptosis, and Ich-1S, which prevents apoptosis. The precise nature of Ich-1 **alternative splicing** and its regulation remain unknown. Here, we show that the production of Ich-1L and Ich-1S transcripts results from alternative exclusion or inclusion of a 61-bp exon. Several splicing factors can regulate Ich-1 splicing. **Serine-arginine-rich** proteins SC35 and ASF/SF2 promote **exon skipping**, decreasing the ratio of Ich-1S to Ich-1L transcripts; whereas heterogeneous nuclear ribonucleoprotein **A1** facilitates exon inclusion, increasing this ratio. Furthermore, in cultured cells, SC35 overexpression increases apoptosis; whereas heterogeneous nuclear ribonucleoprotein **A1** overexpression decreases apoptosis. These results provide the first direct evidence that splicing factors can regulate Ich-1 **alternative splicing** and suggest that **alternative splicing** may be an important regulatory mechanism for apoptosis.

L29 ANSWER 29 OF 73 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 1999063401 MEDLINE
DOCUMENT NUMBER: 99063401 PubMed ID: 9848655
TITLE: Separable roles in vivo for the two RNA binding domains of Drosophila **A1**-hnRNP homolog.
AUTHOR: Zu K; Sikes M L; Beyer A L
CORPORATE SOURCE: Department of Microbiology, University of Virginia, Charlottesville 22908, USA.
CONTRACT NUMBER: GM-39271 (NIGMS)
SOURCE: RNA, (1998 Dec) 4 (12) 1585-98. Journal code: 9509184. ISSN: 1355-8382.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

Search completed by David Schreiber 308-4292

Kam 09/871,809

ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20021218
Entered Medline: 19981229

AB We analyzed the roles of the three domains of a Drosophila hnRNP **A1** homolog by expression of wild-type and mutant versions of HRB87F/hrp36 in Drosophila melanogaster. HRB87F/hrp36 is one of two Drosophila proteins that is most similar to mammalian **A1** hnRNP, and like **A1**, consists of two copies of the RNA-binding domain (RBD) motif followed by a glycine-rich domain (GRD). The role of the domains in nuclear localization and RNA binding to polytene chromosomal sites was determined. RBD-1 and the GRD were largely responsible for both the cellular location of the protein and for the typical chromosomal distribution pattern of the protein at sites of PolII transcription. RBD-1 also provided a role in the **exon-skipping** activity of the protein that was not provided by RBD-2. On the other hand, RBD-2 and the GRD were responsible for the very limited chromosomal distribution pattern seen upon heat shock, when HRB87F/hrp36 is sequestered at heat-shock puff 93D, which encodes a long nucleus-restricted RNA. Thus, these studies indicate that the two RBDs function independently of each other but in concert with the GRD. In addition, the self-association property of the GRD was strikingly evident in these overexpressed proteins.

L29 ANSWER 30 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:226540 HCAPLUS
DOCUMENT NUMBER: 128:320198
TITLE: **Alternative** pre-mRNA **splicing** of the sterol 27-hydroxylase gene (CYP27) caused by a G to A mutation at the last nucleotide of exon 6 in a patient with cerebrotendinous xanthomatosis (CTX)
AUTHOR(S): Chen, Wengen; Kubota, Shunichiro; Seyama, Yousuke
CORPORATE SOURCE: Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: Journal of Lipid Research (1998), 39(3), 509-517
CODEN: JLPRAW; ISSN: 0022-2275
PUBLISHER: Lipid Research, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A recently identified G to A mutation at the last nucleotide of exon 6 of the sterol 27-hydroxylase gene (CYP 27) in a patient with cerebrotendinous xanthomatosis (CTX) was shown here to cause **alternative** pre-mRNA **splicing** of the gene. Northern blot anal. of the patient's RNA revealed a broadened band in the human CYP 27 mRNA region compared to that of the **normal** sample, indicating that there may exist differently spliced mRNA species in the patient. RT-PCR produced three fragments in the patient, one was full-length size and the other two were of smaller sizes. Sequence anal. confirmed that the nucleotide of the full-length size was identical to that of the **normal** full-length cDNA, except for the G to A mutation at codon 362, which corresponds to the last nucleotide of exon 6. One of the smaller size species lacked exon 6 and the other was absent from the 3' terminal 88 bp of exon 6 due to the use of an activated cryptic 5' splice site in exon 6. The correctly spliced mRNA harboring the G to A mutation was responsible for the deficiency of the sterol 27-hydroxylase activity, as confirmed by transfection expt. Transfection of constructed minigenes, with or without the mutation, showed that correctly spliced mRNA was obsd. in the **normal** minigene while the mutant minigene was differently spliced.

Search completed by David Schreiber 308-4292

This is the first report of a G to A substitution at the last nucleotide of an exon resulting in both **normal** and **abnormal** pre-mRNA **splicings**, including **exon skipping** and activating of a coding region cryptic 5' splice site. The results reveal a new mol. basis for the CTX and provide information on **aberrant splicing** of pre-mRNA in multi-exon genes.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 31 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:292807 HCAPLUS
DOCUMENT NUMBER: 129:90895
TITLE: Modification of **alternative splicing** of pre-mRNA by antisense oligonucleotides
AUTHOR(S): Kole, Ryszard
CORPORATE SOURCE: Lineberger Comprehensive Cancer Center and Department of Pharmacology, University of North Carolina, Chapel Hill, NC, 27599, USA
SOURCE: Applied Antisense Oligonucleotide Technology (1998), 451-469. Editor(s): Stein, C. A.; Kreig, Arthur M. Wiley-Liss: New York, N. Y.
CODEN: 65ZQAC
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with several refs. The novel application of antisense oligonucleotides is accomplished by targeting **aberrant splice** sites created by mutations in certain genetic diseases such as thalassemia or **cystic fibrosis**. Blocking these **splice** sites prevents **aberrant splicing** and forces the splicing machinery to reselect correct splice sites thus restoring the proper splicing pathway and the activity of the damaged gene. The first part of this chapter deals with the mechanism of splicing and the suitability of splice sites and other sequence elements as targets for antisense oligonucleotides. The remaing part reviews the use of antisense oligonucleotides as modifiers of splice site selection.
REFERENCE COUNT: 67 THERE ARE 67 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 32 OF 73 MEDLINE
ACCESSION NUMBER: 1998130528 MEDLINE
DOCUMENT NUMBER: 98130528 PubMed ID: 9463322
TITLE: Nearby stop codons in exons of the neurofibromatosis type 1 gene are disparate splice effectors.
AUTHOR: Hoffmeyer S; Nurnberg P; Ritter H; Fahsold R; Leistner W; Kaufmann D; Krone W
CORPORATE SOURCE: Abteilung Humangenetik, Universitat Ulm, Ulm, Germany.. sven.hoffmeyer@medizin.uni-ulm.de
SOURCE: AMERICAN JOURNAL OF HUMAN GENETICS, (1998 Feb) 62 (2) 269-77.
Journal code: 0370475. ISSN: 0002-9297.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 19980416
Entered Medline: 19980406
AB Stop mutations are known to disrupt gene function in different ways. They

both give rise to truncated polypeptides because of the premature-termination codons (PTCs) and frequently affect the metabolism of the corresponding mRNAs. The analysis of neurofibromin transcripts from different neurofibromatosis type 1 (NF1) patients revealed the skipping of exons containing PTCs. The phenomenon of **exon skipping** induced by nonsense mutations has been described for other disease genes, including the **CFTR** (**cystic fibrosis** transmembrance conductance regulator) gene and the fibrillin gene. We characterized several stop mutations localized within a few base pairs in exons 7 and 37 and noticed complete **skipping** of either **exon** in some cases. Because **skipping** of **exon** 7 and of exon 37 does not lead to a frameshift, PTCs are avoided in that way. Nuclear-scanning mechanisms for PTCs have been postulated to trigger the removal of the affected exons from the transcript. However, other stop mutations that we found in either NF1 exon did not lead to a skip, although they were localized within the same region. Calculations of minimum-free-energy structures of the respective regions suggest that both changes in the secondary structure of the mRNA and creation or disruption of exonic sequences relevant for the splicing process might in fact cause these different splice phenomena observed in the NF1 gene.

L29 ANSWER 33 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:179301 SCISEARCH
THE GENUINE ARTICLE: YY818
TITLE: Role of an inhibitory pyrimidine element and polypyrimidine tract binding protein in repression of a regulated alpha-tropomyosin exon
AUTHOR: Gooding C; Roberts G C; Smith C W J (Reprint)
CORPORATE SOURCE: UNIV CAMBRIDGE, DEPT BIOCHEM, 80 TENNIS COURT RD, OLD ADDENBROOKES SITE, CAMBRIDGE CB2 1GA, ENGLAND (Reprint); UNIV CAMBRIDGE, DEPT BIOCHEM, CAMBRIDGE CB2 1GA, ENGLAND
COUNTRY OF AUTHOR: ENGLAND
SOURCE: RNA-A PUBLICATION OF THE RNA SOCIETY, (JAN 1998) Vol. 4, No. 1, pp. 85-100.
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
ISSN: 1355-8382.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 58

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Splicing of exons 2 and 3 of alpha-tropomyosin (TM) involves mutually exclusive selection of either exon 3, which occurs in most cells, or of exon 2 in smooth muscle (SM) cells. The SM-specific selection of exon 2 results from the inhibition of exon 3. At least two essential cis-acting elements are required for exon 3 inhibition, the upstream and downstream regulatory elements (URE and DRE). These elements are essential for repression of TM exon 3 in SM cells, and also mediate a low level of repression of exon 3 in an in vitro 5' splice site competition assay in HeLa extracts. Here, we show that the DRE consists of at least two discrete components, a short region containing a number of UGC motifs, and aln essential pyrimidine-rich tract (DY). We show that the specific sequence of the DY element is important and that DY is able to bind to factors in HeLa nuclear extracts that mediate a low background level of **exon** 3 **skipping**. Deletion of a sequence within DY identified as an optimal binding site for PTB impairs (1) regulation of splicing in vivo, (2) **skipping** of **exon** 3 in an in

vitro 5' splice site competition, (3) the ability of DY competitors to affect the 5' splice site competition in vitro, and (4) binding of PTB to DY. Addition of recombinant PTB to in vitro splicing reactions is able to partially reverse the effects of the DY competitor RNA. The data are consistent with a model for regulation of TM splicing that involves the participation of both tissue-specific and general inhibitory factors and in which PTB plays a role in repressing both splice sites of exon 3.

L29 ANSWER 34 OF 73 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 97449153 MEDLINE
DOCUMENT NUMBER: 97449153 PubMed ID: 9305649
TITLE: The splicing factor **SRp20** modifies splicing of its own mRNA and ASF/SF2 antagonizes this regulation.
AUTHOR: Jumaa H; Nielsen P J
CORPORATE SOURCE: Max Planck Institute for Immunobiology, Freiburg, Germany.
SOURCE: EMBO JOURNAL, (1997 Aug 15) 16 (16) 5077-85.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971017

AB **SRp20** is a member of the highly conserved **SR** family of splicing regulators. Using a variety of reporter gene constructs, we show that **SRp20** regulates **alternative splicing** of its own mRNA. Overexpression of **SRp20** results in a reduction in the level of **exon** 4-**skipped SRp20** transcripts and activates the production of transcripts containing exon 4. These exon 4-included transcripts encode a truncated protein lacking the C-terminal RS domain. We provide evidence that **SRp20** probably enhances the recognition of the otherwise unused, weak splice acceptor of exon 4. The recognition of exons with weak splice acceptor sites may be a general activity of **SRp20**. Unexpectedly, ASF/SF2, another member of the **SR** family, antagonizes the effect of **SRp20** on **SRp20** pre-mRNA splicing and suppresses the production of the exon 4-included form. Our results indicate that ASF/SF2 suppresses the use of the alternative exon 4, most likely by inhibiting the recognition of the splice donor of exon 4. These results demonstrate, for the first time, an auto-regulatory activity of an **SR** protein which is antagonized by a second **SR** protein.

L29 ANSWER 35 OF 73 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 97219967 MEDLINE
DOCUMENT NUMBER: 97219967 PubMed ID: 9121425
TITLE: An intron element modulating 5' splice site selection in the hnRNP **A1** pre-mRNA interacts with hnRNP **A1**.
AUTHOR: Chabot B; Blanchette M; Lapierre I; La Branche H
CORPORATE SOURCE: Departement de Microbiologie, Faculte de Medecine, Universite de Sherbrooke, Quebec, Canada.. b.chabot@courrier.usherb.ca
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1997 Apr) 17 (4) 1776-86.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

Search completed by David Schreiber 308-4292

Kam 09/871,809

FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U65316
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970506
Last Updated on STN: 20021218
Entered Medline: 19970424

AB The hnRNP **A1** pre-mRNA is **alternatively spliced** to yield the **A1** and Alb mRNAs, which encode proteins differing in their ability to modulate 5' splice site selection. Sequencing a genomic portion of the murine **A1** gene revealed that the intron separating exon 7 and the alternative exon 7B is highly conserved between mouse and human. In vitro splicing assays indicate that a conserved element (CE1) from the central portion of the intron shifts selection toward the distal donor site when positioned in between the 5' splice sites of exon 7 and 7B. In vivo, the CE1 element promotes **exon** 7B **skipping**. A 17-nucleotide sequence within CE1 (CE1a) is sufficient to activate the distal 5' splice site. RNase T1 protection/immunoprecipitation assays indicate that hnRNP **A1** binds to CE1a, which contains the sequence UAGAGU, a close match to the reported optimal **A1** binding site, UAGGGU. Replacing CE1a by different oligonucleotides carrying the sequence UAGAGU or UAGGGU maintains the preference for the distal 5' splice site. In contrast, mutations in the AUGAGU sequence activate the proximal 5' splice site. In support of a direct role of the **A1**-CE1 interaction in 5'-splice-site selection, we observed that the amplitude of the shift correlates with the efficiency of **A1** binding. Whereas addition of **SR** proteins abrogates the effect of CE1, the presence of CE1 does not modify U1 snRNP binding to competing 5' splice sites, as judged by oligonucleotide-targeted RNase H protection assays. Our results suggest that hnRNP **A1** modulates splice site selection on its own pre-mRNA without changing the binding of U1 snRNP to competing 5' splice sites.

L29 ANSWER 36 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 97:168375 SCISEARCH
THE GENUINE ARTICLE: WJ164
TITLE: The sex-lethal early splicing pattern uses a default mechanism dependent on the **alternative** 5' **splice** sites
AUTHOR: Zhu C; Urano J; Bell L R (Reprint)
CORPORATE SOURCE: UNIV SO CALIF, DEPT BIOL SCI, PROGRAM MOL BIOL, LOS [illegible] UNIV SO CALIF, DEPT BIOL SCI, PROGRAM MOL BIOL, LOS ANGELES, CA 90089
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (MAR 1997) Vol. 17, No. 3, pp. 1674-1681.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0270-7306.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 57

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Sex-lethal (Sxl) early transcripts have a unique 5' exon and a splicing pattern that differs from that of the late transcripts. While the late transcripts are regulated sex specifically by control of exon 3 inclusion, the early transcripts are not. While the late transcripts include exon 3 by default, the early transcripts **skip**

Search completed by David Schreiber 308-4292

exon 3. Splicing patterns of a reporter gene that mimics the early transcript, and its variants, were analysed in Drosophila transformants and tissue culture cells. The results demonstrate that the early, in contrast to the late, splicing pattern is not regulated by stage-specific or sex-specific trans-acting factors, and so the pattern appears to arise from some type of intrinsic splice site preference or compatibility. Inclusion or exclusion of exon 3 is determined by the identity of the upstream 5' splice site region as late or early. The important region of the early exon lies within 233 nucleotides of the immediately adjacent intron.

L29 ANSWER 37 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 97:669180 SCISEARCH
THE GENUINE ARTICLE: XU421
TITLE: A neuron-specific splicing switch mediated by an array of pre-mRNA repressor sites: Evidence of a regulatory role for the polypyrimidine tract binding protein and a brain-specific PTB counterpart
AUTHOR: Ashiya M; Grabowski P J (Reprint)
CORPORATE SOURCE: UNIV PITTSBURGH, HOWARD HUGHES MED INST, 5TH AVE & RUSKIN ST, PITTSBURGH, PA 15260 (Reprint); UNIV PITTSBURGH, HOWARD HUGHES MED INST, PITTSBURGH, PA 15260; UNIV PITTSBURGH, DEPT SCI BIOL, PITTSBURGH, PA 15260
COUNTRY OF AUTHOR: USA
SOURCE: RNA-A PUBLICATION OF THE RNA SOCIETY, (SEP 1997) Vol. 3, No. 9, pp. 996-1015.
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
ISSN: 1355-8382.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 72
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Tissue-and stage-specific **alternative splicing** events are widespread in mammals, yet the factors and mechanisms that direct these important posttranscriptional events are poorly understood. In this study, we focus on the 24-nt exon of the GABAA receptor gamma 2 pre-mRNA, which is subject to neuron-specific and developmental splicing regulation in the rat cerebellum. Here we show biochemical evidence for a mechanism that directs the selective repression of the neuron-specific

evidence includes the discovery that the pathway of gamma 2 pre-mRNA splicing switches from **exon skipping** to **exon** selection in splicing reactions with a short RNA competitor containing the 3' splice site region upstream of the 24-nt exon. In this assay, exon selection results from the coordinate activation of both flanking introns. A detailed dissection of this pre-mRNA region shows that it contains four repressor sites clustered around the branch site and extending into the 24-nt exon. These repressor sites are pyrimidine rich and bind avidly to the polypyrimidine tract binding protein (PTB) in HeLa nuclear extracts as determined by UV crosslinking/competition assays. Repression of the exon selection pathway is closely associated with the appearance of a specific RNA-protein complex, indicative of an inhibitor complex, that assembles on the repressor array. Upon the switch to the exon selection pathway, a substantial decrease in the inhibitor complex and a reciprocal increase in spliceosome complex A is observed. Excess recombinant PTB squelches the splicing switch and reestablishes **exon skipping** as the predominant splicing pathway. Extracts prepared from rat brain nuclei show

reduced levels of conventional PTB compared to other splicing factors. Nonetheless, the rat brain nuclear extracts contain an activity that assembles an analogous inhibitor complex efficiently. We report a 59-kDa protein, p59, which has an electrophoretic mobility distinct from HeLa and rat kidney PTB, and which behaves in RNA binding assays as if it is the PTB counterpart in rat brain. Evidence that rat brain p59 is structurally related to PTB stems from western blot and immunoprecipitation analysis with a monoclonal antibody specific for the hnRNP I isoform of PTB. A model describing how the repressor array directs coordinate splicing regulation of flanking introns in the context of overlapping positive regulatory elements is discussed. The sequence, (5') UUCUCU (3'), in a pyrimidine context is associated with one class of intron splicing repressor sites that binds PTB in a variety of pre-mRNAs that are regulated by tissue-specific programs.

L29 ANSWER 38 OF 73 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 97318801 MEDLINE
DOCUMENT NUMBER: 97318801 PubMed ID: 9175738
TITLE: A novel mechanism of **aberrant** pre-mRNA **splicing** in humans.
AUTHOR: Cogan J D; Prince M A; Lekhakula S; Bundey S; Futrakul A; McCarthy E M; Phillips J A 3rd
CORPORATE SOURCE: Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232-2578, USA.
CONTRACT NUMBER: DK35592 (NIDDK)
SOURCE: HUMAN MOLECULAR GENETICS, (1997 Jun) 6 (6) 909-12. Journal code: 9208958. ISSN: 0964-6906.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970812
Last Updated on STN: 19970812
Entered Medline: 19970729

AB Eukaryotic pre-mRNA splicing is regulated by consensus sequences at the intron boundaries and branch site. Recently, Sirand-Pugnet et al. reported the importance of an additional intronic sequence, an (A/U)GGG repeat in chicken beta-tropomyosin that is a binding site for a protein required for spliceosome assembly. Interestingly, we have detected mutations in IVS3 of the human growth hormone (GH) gene that affect a [illegible]
In a series of dominant-negative GH mutations that cause **exon skipping**, we found two mutations that do not occur within the 5' and 3' splice sites, or branch consensus sites. The first mutation is a G-->A transition of the 28th base (+28G-->A) of and the second deletes 18 bp (del+28-45) of IVS3 of the human GH gene. These mutations segregated with autosomal dominant GH deficiency in both kindreds and no other allelic GH gene changes were detected. RT-PCR amplification of transcripts from expression vectors containing the +28G-->A or del+28-45 alleles yielded products showing a >10-fold preferred use of **alternative splicing**, similar to findings previously reported for IVS3 donor site mutations. Both mutations are located 28 bp downstream from the 5' splice site and examination of the sequences perturbed revealed an intronic XGGG repeat similar to the repeat found to regulate mRNA splicing in chicken beta-tropomyosin. Interestingly, the XGGG repeats involved in our mutations exhibit homologous spacing to those in a so-called 'winner' RNA sequence. Binding of **A1** heterogeneous nuclear ribonucleoprotein (hnRNP) by 'winner' sequences in

pre-mRNA transcripts is thought to play an important role in pre-mRNA packaging and transport as well as 5' splice site selection in pre-mRNAs that contain multiple 5' splice sites. Our findings suggest that (i) XGGG repeats may regulate **alternative splicing** in the human GH gene and (ii) mutations of these repeats cause GH deficiency by perturbing **alternative splicing**. Mutations of homologous intron sequences may underlie other human diseases.

L29 ANSWER 39 OF 73 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 97240387 MEDLINE
DOCUMENT NUMBER: 97240387 PubMed ID: 9085847
TITLE: A highly stable duplex structure sequesters the 5' splice site region of hnRNP **A1** alternative exon 7B.
AUTHOR: Blanchette M; Chabot B
CORPORATE SOURCE: Department de Microbiologie et Infectiologie, Faculte de Medicine, Universite de Sherbrooke, Quebec, Canada.
SOURCE: RNA, (1997 Apr) 3 (4) 405-19.
Journal code: 9509184. ISSN: 1355-8382.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U65316
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970507
Last Updated on STN: 20021218
Entered Medline: 19970425

AB Exon 7B in the hnRNP **A1** pre-mRNA is **alternatively spliced** to yield **A1** and **A1**(B), two proteins that differ in their ability to modulate 5' splice site selection. Sequencing the murine intron downstream of exon 7B revealed the existence of several regions of similarity to the corresponding human intron. In vitro splicing assays indicate that an 84-nt region (CE6IO) decreases splicing to the proximal 5' splice site in a pre-mRNA carrying the 5' splice sites of exon 7 and 7B. In vivo, the CE6IO element promotes **exon** 7B **skipping** in pre-mRNAs expressed from a mini-gene containing the hnRNP **A1 alternative splicing** unit. Using oligonucleotide-targeted RNase H cleavage assays, we provide support for the existence of highly stable base pairing interactions between CE6IO and the 5' splice site region of exon 7B. Duplex formation occurs in naked pre-mRNA, resists incubation in splicing extracts, and is associated with a reduction in the assembly of U1 snRNP dependent complexes to the 5' splice site of exon 7B. Our results demonstrate that pre-mRNA secondary structure plays an important role in promoting **exon** 7B **skipping** in the **A1** pre-mRNA.

L29 ANSWER 40 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:607867 HCAPLUS
DOCUMENT NUMBER: 127:273673
TITLE: Constitutive APC **exon** 14 **skipping** in early-onset familial adenomatous polyposis reveals a dramatic quantitative distortion of APC gene-specific isoforms
AUTHOR(S): Bala, Shashi; Sulekova, Zora; Ballhausen, Wolfgang G.
CORPORATE SOURCE: Institut fuer Humangenetik der Universitaet, Erlangen, D-91054, Germany
SOURCE: Human Mutation (1997), 10(3), 201-206
CODEN: HUMUE3; ISSN: 1059-7794
PUBLISHER: Wiley-Liss

Search completed by David Schreiber 308-4292

Kam 09/871,809

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Adenomatous polyposis coli (APC) gene transcripts **skipping exon** 14 in combination with the **alternatively spliced** exons 9 and 10A contribute to the heterogeneity of physiol. APC mRNA isoforms. Here the authors report on a novel genotype-phenotype correlation in familial adenomatous polyposis (FAP) with early onset of disease and malignancy due to an APC exon 14 splice defect. Compared to controls, two affected individuals of a FAP kindred presented with a significantly distorted APC mRNA isoform pattern in B lymphocytes. As a result of an A.fwdarw.G transition in the canonical AG-splice acceptor dinucleotide of exon 14, expression levels of all APC mRNA isoforms without exon 14 were dramatically increased and those with exon 14 were simultaneously decreased. **Skipping** of **exon** 14 is a physiol. event also seen in nonmalignant cells, which results in a frameshift to produce low-mol.-wt. APC proteins. Western blot anal. of the patients' lymphoblastoid B cells revealed the identification of intracellularly stable APC protein isoforms with an M, of 55-67 kDa and, thus, the first demonstration of APC proteins encoded by **exon** 14-**skipped** transcripts. The authors postulate that the quant. imbalanced expression of these physiol. APC light chains represents a novel pathogenetic mechanism assocd. with predisposition to FAP.

L29 ANSWER 41 OF 73 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 97174313 MEDLINE
DOCUMENT NUMBER: 97174313 PubMed ID: 9022047
TITLE: Genomic structure and promoter region of the murine Janus-family tyrosine kinase, Jak3.
AUTHOR: Gurniak C B; Thomis D C; Berg L J
CORPORATE SOURCE: Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA.
SOURCE: DNA AND CELL BIOLOGY, (1997 Jan) 16 (1) 85-94.
Journal code: 9004522. ISSN: 1044-5498.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970306
Last Updated on STN: 19970306
Entered Medline: 19970227

AB Genomic DNA sequences encoding the murine Janus family tyrosine kinase Jak3 were isolated to determine the intron-exon structure of the gene and to investigate the phylogeny of Jak-family kinases. The murine Jak3 gene comprises approximately 15 kbp of genomic DNA and consists of 23 exons. The organization of sequences encoding the pseudo-kinase domain of Jak3 is similar to the intron-exon structure encoding catalytic domains of **Src**-family tyrosine kinases, whereas the pattern of introns-exons encoding the Jak3 kinase domain shows no structural similarity to that of other tyrosine kinase genes. Genomic analysis further indicates that **alternative splicing** gives rise to different forms of the murine Jak3 mRNA encoding different isoforms of the Jak3 protein. Analysis of Jak3 intron-exon structure also suggests that a mutation in the human JAK3 gene responsible for a severe combined immune deficiency (SCID) phenotype results from **aberrant splicing** of the JAK3 transcript. Finally, potential regulatory sequences in the upstream region of the murine Jak3 gene were analyzed and are discussed in relation to the known expression pattern of Jak3.

Search completed by David Schreiber 308-4292

Kam 09/871,809

L29 ANSWER 42 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 96:551567 SCISEARCH
THE GENUINE ARTICLE: UY025
TITLE: MUSCLE-SPECIFIC SPLICING ENHANCERS REGULATE INCLUSION OF THE CARDIAC TROPONIN-T ALTERNATIVE EXON IN EMBRYONIC SKELETAL-MUSCLE
AUTHOR: RYAN K J; COOPER T A (Reprint)
CORPORATE SOURCE: BAYLOR COLL MED, DEPT PATHOL, HOUSTON, TX, 77030 (Reprint); BAYLOR COLL MED, DEPT PATHOL, HOUSTON, TX, 77030; BAYLOR COLL MED, DEPT CELL BIOL, HOUSTON, TX, 77030
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (AUG 1996) Vol. 16, No. 8, pp. 4014-4023.
ISSN: 0270-7306.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 64

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The alternative exon 5 of the striated muscle-specific cardiac troponin T (cTNT) gene is included in mRNA from embryonic skeletal and cardiac muscle and excluded in mRNA from the adult, The embryonic splicing pattern is reproduced in primary skeletal muscle cultures for both the endogenous gene and transiently transfected minigenes, whereas in nonmuscle cell lines, minigenes express a default **exon skipping** pattern, Using this experimental system, we previously showed that a purine-rich **splicing** enhancer in the **alternative** exon functions as a constitutive splicing element but not as a target for factors regulating cell-specific splicing, In this study, we identify four intron elements, one located upstream and three located downstream of the alternative exon, which act in a positive manner to mediate the embryonic splicing pattern of exon inclusion. Synergistic interactions between at least three of the four elements are necessary and sufficient to regulate **splicing** of a heterologous **alternative** exon and heterologous **splice** sites, Mutations in these elements prevent activation of exon inclusion in muscle cells but do not affect the default level of exon inclusion in nonmuscle cells. Therefore, these elements function as muscle specific splicing enhancers (MSEs) and are the first muscle-specific positive-acting splicing elements to be described. One MSE located downstream from the alternative exon is conserved in the rat and chicken cTNT genes, A related sequence is found in a third muscle-specific [illegible] exon with a developmental pattern of **alternative splicing** similar to that of rat and chicken cTNT. Therefore, the MSEs identified in the cTNT gene may play a role in developmentally regulated **alternative splicing** in a number of different genes.

L29 ANSWER 43 OF 73 MEDLINE
ACCESSION NUMBER: 96407015 MEDLINE
DOCUMENT NUMBER: 96407015 PubMed ID: 8811102
TITLE: A short CIC-2 mRNA transcript is produced by **exon skipping**.
AUTHOR: Chu S; Murray C B; Liu M M; Zeitlin P L
CORPORATE SOURCE: Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD 21287-2533, USA.
CONTRACT NUMBER: R29 HL48274 (NHLBI)
SOURCE: NUCLEIC ACIDS RESEARCH, (1996 Sep 1) 24 (17) 3453-7.
Journal code: 0411011. ISSN: 0305-1048.

Search completed by David Schreiber 308-4292

Kam 09/871,809

PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF005720
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961125

AB CIC-2 is a voltage- and volume-regulated chloride channel expressed in many tissues. We have shown that CIC-2 in rat lung airways is significantly down-regulated after birth [Murray,C.B. et al. (1995) Am. J. Respir. Cell Mol. Biol., 12, 597-604]. During PCR amplification from rat lung cDNA, a second transcript was identified which is 60 bp shorter than the full length sequence. The peptide translated from this 60 bp sequence contains many positively charged amino acid residues. Rat genomic DNA sequencing showed that the 60 bp sequence is an intact exon. A 71% pyrimidine content and an AAG 3'-end splice site in the intron immediately upstream from the 60 bp sequence were identified which may account for the **alternative splicing** of the following exon. Human genomic sequence analyses demonstrated similar intron-exon arrangement. A high CT content and an AAG 3' acceptor site were conserved in the intron corresponding to the rat upstream intron. The presence of the full length short form transcript was confirmed in rat kidney by RT-PCR, and the ratio of the long and the short form transcripts varied significantly according to the tissues examined, with the lowest long/short form ratio found in the lung among the tissues studied. Our data demonstrated that the **alternatively spliced** short form (CIC-2S) is transcribed in many rat tissues, the ratio of the long/short form transcripts is lower in the lung compared with the brain, and the genomic organization in this area is conserved in rat and human.

L29 ANSWER 44 OF 73 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 96189114 MEDLINE
DOCUMENT NUMBER: 96189114 PubMed ID: 8628299
TITLE: Interactions among **SR** proteins, an exonic splicing enhancer, and a lentivirus Rev protein regulate **alternative splicing.**
AUTHOR: Gontarek R R; Derse D
CORPORATE SOURCE: Laboratory of Leukocyte Biology, National Cancer Institute-Frederick Cancer Research and Development Center,
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1996 May) 16 (5) 2325-31. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960708
Last Updated on STN: 19980206
Entered Medline: 19960621

AB We examine here the roles of cellular splicing factors and virus regulatory proteins in coordinately regulating **alternative splicing** of the tat/rev mRNA of equine infectious anemia virus (EIAV). This bicistronic mRNA contains four exons; exons 1 and 2 encode Tat, and exons 3 and 4 encode Rev. In the absence of Rev expression, the four-exon mRNA is synthesized exclusively, but when Rev is expressed, **exon** 3 is **skipped** to produce an mRNA that contains only

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exons 1, 2, and 4. We identify a purine-rich exonic splicing enhancer (ESE) in exon 3 that promotes exon inclusion. Similar to other cellular ESEs that have been identified by other laboratories, the EIAV ESE interacted specifically with **SR** proteins, a group of **serine/arginine-rich** splicing factors that function in constitutive and **alternative** mRNA **splicing**. Substitution of purines with pyrimidines in the ESE resulted in a switch from **exon** inclusion to **exon skipping** in vivo and abolished binding of **SR** proteins in vitro. **Exon skipping** was also induced by expression of EIAV Rev. We show that Rev binds to exon 3 RNA in vitro, and while the precise determinants have not been mapped, Rev function in vivo and RNA binding in vitro indicate that the RNA element necessary for Rev responsiveness overlaps or is adjacent to the ESE. We suggest that EIAV Rev promotes **exon skipping** by interfering with **SR** protein interactions with RNA or with other splicing factors.

L29 ANSWER 45 OF 73 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 20
ACCESSION NUMBER: 1996:707230 HCAPLUS
DOCUMENT NUMBER: 126:5750
TITLE: Combination of two mutant alpha spectrin alleles underlies a severe spherocytic hemolytic anemia
AUTHOR(S): Wichterle, Hynek; Hanspal, Manjit; Palek, Jiri; Jarolim, Petr
CORPORATE SOURCE: Department of Biomedical Research, St. Elizabeth's Medical Center, Boston, MA, 02135, USA
SOURCE: Journal of Clinical Investigation (1996), 98(10), 2300-2307
CODEN: JCINAO; ISSN: 0021-9738
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors studied a patient with a severe spherocytic hemolytic anemia without family history of spherocytosis. Anal. of patient's erythrocyte membrane proteins revealed spectrin deficiency and a truncated .alpha. spectrin protein. The authors detd. that the patient is a compd. heterozygote with two mutations in .alpha. spectrin gene. Mutation in the paternal allele, designated .alpha. spectrinPRAGUE, is a transition A to G in the penultimate position of intron 36 that leads to **skipping** of **exon** 37, frameshift, and prodn. of the truncated .alpha. spectrin protein. The maternal allele, designated .alpha. spectrinLEPRA, -99 of intron 30. This mutation enhances an **alternative** acceptor **splice** site 70 nucleotides upstream from the regular site. The **alternative splicing** causes a frameshift and premature termination of translation leading to a significant decrease in a spectrin prodn. The .alpha.LEPRA mutation is linked to a spectrin .alpha.IIa marker that was assocd. with recessive or nondominant spectrin-deficient hereditary spherocytosis in .apprx.50% of studied families. The authors conclude that the .alpha.LEPRA mutation combined in trans with the .alpha.PRAGUE mutation underlies the severe hemolytic anemia in the proband. The authors suggest that allele .alpha. spectrinLEPRA may be frequently involved in pathogenesis of recessive or nondominant spectrin-deficient hereditary spherocytosis.

L29 ANSWER 46 OF 73 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96326296 EMBASE
DOCUMENT NUMBER: 1996326296
TITLE: **Exon** 10 **skipping** caused by intron 10

Search completed by David Schreiber 308-4292

splice donor site mutation in cholesteryl ester transfer protein gene results in **abnormal** downstream **splice** site selection.

AUTHOR: Sakai N.; Santamarina-Fojo S.; Yamashita S.; Matsuzawa Y.; Brewer H.B. Jr.

CORPORATE SOURCE: Molecular Disease Branch, National Heart, Lung/Blood Institute, National Institutes of Health, 10 Center Drive,Bethesda, MD 20892-1666, United States

SOURCE: Journal of Lipid Research, (1996) 37/10 (2065-2073).
ISSN: 0022-2275 CODEN: JLPRAW

COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Cholesteryl ester transfer protein (CETP) deficiency is the most common cause of hyperalphalipoproteinemia in Japan. However, the genetic basis of this disorder has not been fully characterized. We have studied a 49-year-old Japanese male presenting with total cholesterol, HDL-cholesterol, and apolipoprotein A-I levels of 300, 236, and 233 mg/dl, respectively, and total absence of CETP activity and mass in plasma. Sequence analysis of the patient's CETP gene revealed that the splice donor consensus GT was substituted by GG in intron 10 (intron 10 splice defect) and by AT in intron 14 (intron 14 splice defect). Restriction digestion of PCR-amplified DNA using NdeI and MaeIII established that the patient was a compound heterozygote for both gene defects. Sequencing of cDNA amplified by RT-PCR from the patient's monocyte-derived macrophage RNA demonstrated **abnormal splicing** with deletion of exon 10 as well as **alternative splicing** at a native AG site located 31 nucleotides 5' of the **normal** splice acceptor in intron 13. Thus, the intron 10 splice defect results in **exon** 10 **skipping** and the insertion of a 31 bp fragment between exon 13 and exon 14, which contains an in frame stop codon. The presence of abnormally spliced mRNA was further confirmed by amplification of patient cDNA using CETP specific primers. **Abnormal splicing** of exon 14 as a result of the intron 14 splice defect was not detected, indicating potential unstable CETP mRNA derived from that mutation. These findings demonstrate that a novel splice site mutation in intron 10 of the CETP gene results in the **skipping** of **exon** 10, as well as disruption of' downstream splicing at intron 13 identifying a novel mechanism leading to

L29 ANSWER 47 OF 73 EMBASE

ACCESSION NUMBER: 96340261 EMBASE
DOCUMENT NUMBER: 1996340261

TITLE: Three novel mutations of the NF1 gene detected by temperature gradient gel electrophoresis of exons 5 and 8.

AUTHOR: Horn D.; Robinson P.N.; Boddrich A.; Buske A.; Tinschert S.; Nurnberg P.

CORPORATE SOURCE: Institut fur Medizinische Genetik, Universitatsklinikum Charite,D-10098 Berlin, Germany

SOURCE: Electrophoresis, (1996) 17/10 (1559-1563).
ISSN: 0173-0835 CODEN: ELCTDN

COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
012 Ophthalmology
013 Dermatology and Venereology

Search completed by David Schreiber 308-4292

Kam 09/871,809

022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We screened a total of 100 unrelated patients with neurofibromatosis type 1 (NF1) for mutations in exons 5 and 8 of the NF1 gene using temperature gradient gel electrophoresis (TGGE). Careful interpretation of exon 5 TGGE patterns was necessary due to interference by an exonic polymorphism. Three novel mutations were identified: a stop mutation in exon 5 (Q239X) caused by a **C.fwdarw.T** transition at cDNA nucleotide position 715, a transition at the invariant G of the splice acceptor site in intron 4c (G655-1A), and a transversion at the invariant G of the splice donor site in intron 8 (G1188S+1T). Analysis of mRNA revealed the predicted **abnormal splice** products. While **skipping** of **exon** 5 causes a shift in the reading frame with a premature stop codon downstream in the middle of **exon** 6, **skipping** of **exon** 8 leads to an in-frame deletion with the predicted protein product being shortened by 41 amino acids.

L29 ANSWER 48 OF 73 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 97015883 MEDLINE
DOCUMENT NUMBER: 97015883 PubMed ID: 8862520
TITLE: Altered levels of the Drosophila HRB87F/hrp36 hnRNP protein have limited effects on **alternative splicing** in vivo.
AUTHOR: Zu K; Sikes M L; Haynes S R; Beyer A L
CORPORATE SOURCE: Department of Microbiology, University of Virginia, Charlottesville 22908, USA.
CONTRACT NUMBER: GM-39271 (NIGMS)
SOURCE: MOLECULAR BIOLOGY OF THE CELL, (1996 Jul) 7 (7) 1059-73. Journal code: 9201390. ISSN: 1059-1524.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961209

AB The Drosophila melanogaster genes Hrb87F and Hrb98DE encode the fly proteins HRB87F and HRB98DE (also known as hrp36 and hrp38, respectively) that are most similar in sequence and function to mammalian A/B-type hnRNP proteins. Using overexpression and deletion mutants tested the hypothesis that the ratio of A/B hnRNP proteins to **SR** family proteins modulates certain types of **alternative splice**-site selection. In flies in which HRB87F/hrp36 had been overexpressed 10- to 15-fold above **normal** levels, aberrant internal **exon skipping** was induced in at least one endogenous transcript, the dopa decarboxylase (Ddc) pre-mRNA, which previously had been shown to be similarly affected by excess HRB98DE/hrp38. In a second endogenous pre-mRNA, excess HRB87F/hrp36 had no effect on **alternative** 3' **splice**-site selection, as expected from mammalian hnRNP studies. Immunolocalization of the excess hnRNP protein showed that it localized correctly to the nucleus, specifically to sites on or near chromosomes, and that the peak of **exon-skipping** activity in Ddc RNA correlated with the peak of chromosomally associated hnRNP protein. The chromosomal association and level of the **SR** family of proteins were not significantly affected by the large increase in hnRNP proteins during this time period. Although these results are consistent with a possible role

Search completed by David Schreiber 308-4292

for hnRNP proteins in **alternative splicing**, the more interesting finding was the failure to detect significant adverse effects on flies with a greatly distorted ratio of hnRNPs to **SR** proteins. Electron microscopic visualization of the general population of active genes in flies overexpressing hnRNP proteins also indicated that the great majority of genes seemed **normal** in terms of cotranscriptional RNA processing events, although there were a few abnormalities consistent with rare **exon-skipping** events. Furthermore, in a Hrb87F null mutant, which is viable, the **normal** pattern of Ddc **alternative splicing** was observed, indicating that HRB87F/hrp36 is not required for Ddc splicing regulation. Thus, although splice-site selection can be affected in at least a few genes by gross overexpression of this hnRNP protein, the combined evidence suggests that if it plays a general role in **alternative splicing** in vivo, the role can be provided by other proteins with redundant functions, and the role is independent of its concentration relative to **SR** proteins.

L29 ANSWER 49 OF 73 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 96184847 MEDLINE
DOCUMENT NUMBER: 96184847 PubMed ID: 8604331
TITLE: During in vivo maturation of eukaryotic nuclear mRNA, splicing yields excised exon circles.
AUTHOR: Bailleul B
CORPORATE SOURCE: Unite 124 INSERM, Institut de Recherches sur le Cancer de Lille, France.
SOURCE: NUCLEIC ACIDS RESEARCH, (1996 Mar 15) 24 (6) 1015-9. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 19960524
Last Updated on STN: 19960524
Entered Medline: 19960516

AB Circular splicing has already been described on nuclear pre-mRNA for certain splice sites far apart in the multi exonic ETS-1 gene and in the single 1.2 kb exon of the **Sry** locus. To date, it is unclear how splice site juxtaposition occurs in **normal** and circular splicing. The splice site selection of an internal exon is likely to [illegible] predict that, albeit at low frequency, internal exons yield circular RNA by **splicing** as an **error**-prone mechanism of exon juxtaposition or, perhaps more interestingly, as a regulated mechanism on alternative exons. To address this question, the circular exon formation was analyzed at three ETS-1 internal exons (one **alternative spliced** exon and two constitutive), in human cell line and blood cell samples. Here, we show by RT-PCR and sequencing that exon circular splicing occurs at the three individual exons that we examined. RNase protection experiments suggest that there is no correlation between exon circle expression and **exon skipping**.

L29 ANSWER 50 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:691173 HCAPLUS
DOCUMENT NUMBER: 125:325288
TITLE: **Alternative splicing** of exon 12 of the COL2A1 gene interrupts the triple helix of type-II collagen in the Kniest form of spondyloepiphyseal

Kam 09/871,809

dysplasia
AUTHOR(S): Chen, Luping; Yang, Winnie; Cole, William G.
CORPORATE SOURCE: Research Institute, Hospital Sick Children, Toronto, ON, M5G 1X8, Can.
SOURCE: Journal of Orthopaedic Research (1996), 14(5), 712-721
CODEN: JOREDR; ISSN: 0736-0266
PUBLISHER: Journal of Bone and Joint surgery, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An autosomal dominant mutation in the COL2A1 gene was identified in a child with the Kniest form of spondyloepiphyseal dysplasia. A C to T transition at nucleotide 35 of exon 12 changed the codon GCG for alanine 102 of the triple helical domain of .alpha.1(II) chains of type-II collagen to GTG for valine. The transition also introduced a GT dinucleotide into exon 12. Anal. of cDNA prepd. from Kniest cartilage showed that in vivo the transition resulted in an **alternatively spliced** mRNA that lacked the 21 3' nucleotides from exon 12. The cartilage cDNA contained approx. equal amts. of **normal** cDNA and shortened mutant cDNA. The deletion of 21 nucleotides from the mutant cDNA maintained the translational reading frame but resulted in the loss of alanine 102 to lysine 108, which interrupted the repetitive glycine-X-Y triplet sequence required for formation of the triple helix. Type-II collagen mols. contg. one or more mutant chains were expected, therefore, to contain interrupted triple helixes with a short amino-terminal helical domain A and a large carboxy-terminal helical domain B. Kniest cartilage contained a reduced amt. of pepsin-solubilized type-II collagen that consisted of overmodified .alpha.1(II) chains. Peptide mapping showed that the over-modifications extended to the carboxy terminus of the .alpha.1(II) chains. Pepsin digestion also yielded shortened .alpha.1(II) chains corresponding to helical domain B. Kniest chondrocytes cultured in alginate beads produced type-II collagen that was not stably incorporated into the pericellular matrix. This study highlights the importance of dominant neg. mutations of COL2A1 in producing Kniest dysplasia.

L29 ANSWER 51 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 96:605666 SCISEARCH
THE GENUINE ARTICLE: VB557
TITLE: ESSENTIAL NUCLEOTIDES DIRECT NEURON-SPECIFIC SPLICING OF GAMMA(2) PRE-MESSENGER-RNA
AUTHOR: ZHANG L; ASHIYA M; SHERMAN T G; GRABOWSKI P J (Reprint)
CORPORATE SOURCE: UNIV PITTSBURGH, HOWARD HUGHES MED INST, PITTSBURGH, PA, [illegible] PITTSBURGH, PA, 15260; UNIV PITTSBURGH, DEPT BIOL SCI, PITTSBURGH, PA, 15260; UNIV PITTSBURGH, DEPT NEUROSCI, PITTSBURGH, PA, 15260
COUNTRY OF AUTHOR: USA
SOURCE: RNA-A PUBLICATION OF THE RNA SOCIETY, (JUL 1996) Vol. 2, No. 7, pp. 682-698.
ISSN: 1355-8382.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 41
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Tissue- and stage-specific pre-mRNA splicing events are prevalent in mammals, yet molecular details are lacking about these important mechanisms of posttranscriptional gene control. In this study, we investigate the regulated splicing of rat gamma 2 pre-mRNA, a subunit of the GABA, receptor, as a step toward understanding the molecular basis of

Search completed by David Schreiber 308-4292

a neuron-specific splicing event involving cassette exon selection, cell- and substrate-specific regulation of gamma 2 pre-mRNA is recapitulated in a neuronal cell line derived from the cerebellum, which produces enhanced levels of the exon-selected mRNA. In contrast, a control cell line derived from non-neuronal cells of the pituitary produces prominent levels of the unregulated, **exon-skipped** mRNA. The cerebellar and pituitary cell lines are well matched in overall splicing efficiency and produce an invariant pattern of splicing for a control substrate, which is **alternatively spliced** but not regulated in this system. The appropriateness of the two cell lines is indicated by an extended mRNA mapping experiment, which documents the region-specific switch in exon selection throughout rat brain. Using this pair of cell lines, we show that large intron segments flanking the regulated exon are dispensable for regulation. These intron regions have been deleted to generate a minimal splicing substrate for the purpose of identifying essential RNA elements. In this context, we show that essential nucleotides are located at positions +7, +8, and +9 of the regulated exon and in a 9-nt adenosine-rich region of the adjacent 3' splice site. Due to the proximity and base complementarity of the required nucleotides, experiments were devised to test models involving the recognition of two single-stranded signals, or one duplex RNA signal. These results clearly disfavor the duplex RNA recognition model and indicate that the required regions are recognized as independent, single strands in neuronal cells. A weak 5' splice site adjacent to the regulated exon is required as a third essential element. Although the importance of a weak 5' splice site is common to other regulated systems such as NCAM, the essential nucleotides in the exon and 3' splice site region defined in this study for gamma 2 splicing regulation are novel.

L29 ANSWER 52 OF 73 MEDLINE

ACCESSION NUMBER:	97001219 MEDLINE
DOCUMENT NUMBER:	97001219 PubMed ID: 8844211
TITLE:	Complex **cystic fibrosis** allele R334W-R1158X results in reduced levels of correctly processed mRNA in a pancreatic sufficient patient.
AUTHOR:	Duarte A; Amaral M; Barreto C; Pacheco P; Lavinha J
CORPORATE SOURCE:	Departamento de Genetica Humana, Instituto Nacional de Saude Dr. Ricardo Jorge, Lisboa, Portugal.
SOURCE:	HUMAN MUTATION, (1996) 8 (2) 134-9. Journal code: 9215429. ISSN: 1059-7794.
PUB. COUNTRY:	United States
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	199612
ENTRY DATE:	Entered STN: 19970128 Last Updated on STN: 19970128 Entered Medline: 19961224

AB **CFTR** alleles containing two mutations have been very rarely found in **cystic fibrosis** (CF) patients. They provide an opportunity to study the effect of two in cis-interacting gene defects on gene expression. Here, we describe a three-generation CF family with a complex **CFTR** allele that has not been previously described, containing the missense mutation R334W in exon 7 and the nonsense mutation R1158X in exon 19. Lymphocyte RNA analysis showed that (1) the mRNA corresponding to the complex allele is present although at markedly reduced levels; and (2) the nonsense mutation does not lead to detectable **skipping** of **exon** 19. The clinical picture of the patients with the genotype R334W-R1158X/delta F508 is characterized by

pancreatic sufficiency and an atypical course of the disease.

L29 ANSWER 53 OF 73 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 96437395 MEDLINE
DOCUMENT NUMBER: 96437395 PubMed ID: 8840112
TITLE: Analysis of **alternative splicing** patterns in the **cystic fibrosis** transmembrane conductance regulator gene using mRNA derived from lymphoblastoid cells of **cystic fibrosis** patients.
AUTHOR: Bienvenu T; Beldjord C; Chelly J; Fonknechten N; Hubert D; Dusser D; Kaplan J C
CORPORATE SOURCE: Laboratoire de Biochimie Genetique, CHU Cochin, Paris, France.
SOURCE: EUROPEAN JOURNAL OF HUMAN GENETICS, (1996) 4 (3) 127-34. Journal code: 9302235. ISSN: 1018-4813.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970102

AB Using in vitro amplification of cDNA by the polymerase chain reaction, we analyzed **alternatively spliced** events of **cystic fibrosis** transmembrane conductance regulator gene in lymphoblastoid cells. Ten **alternatively spliced** transcripts were identified using analysis of 6 overlapping segments of amplified cDNA, 4 of which have not been described previously. These include transcripts lacking exon 16, 17b, 22 and a transcript resulting from the use of a cryptic acceptor and donor splice sites. Moreover, in 2 **cystic fibrosis** (CF) patients bearing nonsense mutations E60X or W1282X, we observed that nonsense mutations are associated with an alteration of splice site selection in vivo resulting in **exon skipping** of constitutive exons or in the use of cryptic splice sites. In addition, even though lymphoblastoid cells are not the relevant tissue to address the question of the relationship between clinical respiratory phenotype and genotype, our results concerning adult CF patients (delta F508/ delta F508) suggest that individual-specific RNA splicing patterns could influence the severity of the CF pulmonary

events proves to be significant in CF and to be a common feature of disease genes, the study of RNA splicing could become an important tool for the analysis of the genotype-phenotype relationship in many inherited disorders.

L29 ANSWER 54 OF 73 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:37081 BIOSIS
DOCUMENT NUMBER: PREV199698609216
TITLE: A recurring dominant negative mutation causes autosomal dominant growth hormone deficiency-A clinical research center study.
AUTHOR(S): Cogan, Joy D. (1); Ramel, Bjorn; Lehto, Markku; Phillipsi, John Ii; Prince, Melissa; Blizzard, Robert M.; De Ravel, Thomy J. L.; Brammert, Margareta; Groop, Leif
CORPORATE SOURCE: (1) Div. Genetics, Vanderbilt Univ. Sch. Med., DD-2205 Med. Cent. North, Nashville, TN 37232-2578 USA
SOURCE: Journal of Clinical Endocrinology & Metabolism, (1995) Vol.

Search completed by David Schreiber 308-4292

80, No. 12, pp. 3591-3595.
ISSN: 0021-972X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Familial isolated GH deficiency type II (IIGHD-II) is an autosomal dominant disorder that has been previously shown in some patients to be caused by heterogeneous GH gene defects that affect GH messenger RNA (mRNA) splicing. We report here our finding of multiple G fwdarw A transitions of the first base of the donor splice site of IVS 3 (+1G fwdarw A) in IGHD II subjects from three nonrelated kindreds from Sweden, North America, and South Africa. This +1G fwdarw A substitution creates an NlaIII site that was used to demonstrate that all affected individuals in all three families were heterozygous for the mutation. To determine the effect of this mutation on GH mRNA processing, HeLa cells were transfected with expression plasmids containing **normal** or mutant +1G fwdarw A alleles, and complementary DNAs from the resulting GH mRNAs were sequenced. The mutation was found to destroy the GH IVS 3 donor splice site, causing **skipping** of **exon** 3 and loss of the codons for amino acids 32-71 of the mature GH peptide from the mutant GH mRNA. Our finding of **exon** 3 **skipping** in transcripts of the + 1G fwdarw A mutant allele is identical to our previous report of a different sixth base **transition** (+6T fwdarw C) **mutation** of the IVS 3 donor splice site that also causes IGHD II. Microsatellite analysis of an affected subjects' DNA from each of the three nonrelated kindreds indicates that the +1G fwdarw A mutation arose independently in each family. Finding that neither grandparent has the mutation in the first family suggests that it arose de novo in that family. Our data indicate that 1) +1G fwdarw A IVS 3 mutations perturb GH mRNA splicing and cause IGHD II; and 2) these mutations can present as de novo GHD cases.

L29 ANSWER 55 OF 73 EMBASE

ACCESSION NUMBER: 95199715 EMBASE
DOCUMENT NUMBER: 1995199715

TITLE: Occurrence of multiple aberrantly spliced mRNAs of the LDL-receptor gene upon a donor splice site mutation that causes familial hypercholesterolemia (FH(Benevento)).

AUTHOR: Lelli N.; Garuti R.; Ghisellini M.; Tiozzo R.; Rolleri M.; Aimale V.; Ginocchio E.; Naselli A.; Bertolini S.; Calandra S.
CORPORATE SOURCE: Sezione di Patologia Generale, Dipartimento di Scienze Biomediche, Universita di Modena, Via Campi 287,41100 [illegible]

SOURCE: Journal of Lipid Research, (1995) 36/6 (1315-1324).
ISSN: 0022-2275 CODEN: JLPRAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 007 Pediatrics and Pediatric Surgery
018 Cardiovascular Diseases and Cardiovascular Surgery
022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A novel point mutation of the LDL-receptor gene was found in an Italian patient with homozygous familial hypercholesterolemia. The SSCP analysis of the promoter and of 16 out of the 18 exons of the LDL-receptor gene was negative, suggesting that the mutation might be located in the region of the gene encompassing exons 14 and 15, a region that had not been amenable to polymerase chain reaction (PCR) amplification from genomic DNA. This region was amplified from cDNA by reverse transcription PCR (RT-PCR).

RT-PCR of proband cDNA generated three fragments of 800, 600, and 550 bp, respectively, as opposed to a single 720 bp fragment obtained from control cDNA. The sequence of these fragments showed that: i) in the 800-bp fragment exon 14 continued with the 5' end of intron 15 (90 nucleotides), which in turn was followed by exon 16; ii) in the 600-bp fragment exon 14 was followed by the 5' end of exon 15 (50 nucleotides), which continued with exon 16; iii) in the 550-bp fragment exon 14 joined directly to exon 16. These abnormally spliced mRNAs resulted from a G.fwdarw.A transition at the +1 nucleotide of intron 15, which changed the invariant GT dinucleotide- of the 5' donor splice site. That was associated with the activation of two cryptic donor splice sites in intron 15 and exon 15, respectively, and the use of an **alternative splicing** leading to the **skipping** of **exon** 15. Northern blot analysis showed that the overall content of these aberrantly spliced mRNAs in proband fibroblasts was one-fourth that found in control cells. These abnormally spliced mRNAs are predicted to encode three abnormal receptor proteins: the first would contain an insertion of 30 novel amino acids; the second would be a truncated protein of 709 amino acids; the third would be devoid of the 57 amino acids of the O- linked sugar domain. Ligand blot experiments indicated that the amount of LDL-receptor present in proband's fibroblasts was approximately one-tenth that found in control cells.

L29 ANSWER 56 OF 73 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 24
ACCESSION NUMBER: 1995:857358 HCAPLUS
DOCUMENT NUMBER: 124:172584
TITLE: A single point mutation in the splice donor site of the low-density-lipoprotein-receptor gene produces intron read-through, **exon-skipped** and cryptic-site-utilized transcripts
AUTHOR(S): Maruyama, Takao; Miyake, Yasuko; Tajima, Shoji; Funahashi, Tohru; Matsuzawa, Yuji; Yamamoto, Akira
CORPORATE SOURCE: Dep. Etiol. Pathophysiol., Natl. Cardiovascular Cent. Res. Inst., Osaka, Japan
SOURCE: European Journal of Biochemistry (1995), 232(3), 700-5
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Springer
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Familial hypercholesterolemia is a genetic disorder caused by mutations of the low-d.-lipoproteins (LDL) receptor gene. The structures of LDL receptor mRNA transcripts were characterized in the fibroblasts of a homozygous patient carrying a single base substitution (**T . fwdarw. C**) at the 5' splice donor site of intron 12 of the LDL receptor gene. Three aberrant transcripts were identified as a consequence of intron-12 read-through, **exon**-12 **skipping** and utilization of a cryptic splice donor site. Only a point mutation at the 5' splice donor site caused the prodn. of 3 **alternatively spliced** products. None of these transcripts produced a functional LDL receptor protein in this patient.

L29 ANSWER 57 OF 73 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 95220657 MEDLINE
DOCUMENT NUMBER: 95220657 PubMed ID: 7535716
TITLE: Mutations in the unc-52 gene responsible for body wall muscle defects in adult Caenorhabditis elegans are located in **alternatively spliced** exons.
AUTHOR: Rogalski T M; Gilchrist E J; Mullen G P; Moerman D G
CORPORATE SOURCE: Department of Zoology, University of British Columbia,

Vancouver, Canada.
SOURCE: GENETICS, (1995 Jan) 139 (1) 159-69.
Journal code: 0374636. ISSN: 0016-6731.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950518
Last Updated on STN: 19990129
Entered Medline: 19950511

AB The unc-52 gene in Caenorhabditis elegans produces several large proteins that function in the basement membrane underlying muscle cells. Mutations in this gene result in defects in myofilament assembly and in the attachment of the myofilament lattice to the muscle cell membrane. The st549 and ut111 alleles of unc-52 produce a lethal (Pat) terminal phenotype whereas the **e444**, e669, e998, e1012 and e1421 mutations result in viable, paralyzed animals. We have identified the sequence alterations responsible for these mutant phenotypes. The st549 allele has a premature stop codon in exon 7 that should result in the complete elimination of unc-52 gene function, and the ut111 allele has a Tc1 transposon inserted into the second exon of the gene. The five remaining mutations are clustered in a small interval containing three adjacent, **alternatively spliced** exons (16, 17 and 18). These mutations affect some, but not all of the unc-52-encoded proteins. Thirteen intragenic revertants of the e669, e998, e1012 and e1421 alleles have also been sequenced. The majority of these carry the original mutation plus a G to A transition in the conserved splice acceptor site of the affected exon. This result suggests that reversion of the mutant phenotype in these strains may be the result of **exon-skipping**.

L29 ANSWER 58 OF 73 MEDLINE DUPLICATE 26

ACCESSION NUMBER: 94316610 MEDLINE
DOCUMENT NUMBER: 94316610 PubMed ID: 8041722
TITLE: The **A1** and A1B proteins of heterogeneous nuclear ribonucleoparticles modulate 5' splice site selection in vivo.
AUTHOR: Yang X; Bani M R; Lu S J; Rowan S; Ben-David Y; Chabot B
CORPORATE SOURCE: Departement de Microbiologie, Faculte de Medecine, Universite de Sherbrooke, PQ Canada.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Jul 19) 91 (15) 6924-8.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X78885
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940905
Last Updated on STN: 20021218
Entered Medline: 19940822

AB Recent in vitro results suggest that the heterogeneous nuclear ribonucleoparticle (hnRNP) **A1** protein modulates **alternative splicing** by favoring distal 5' splice site (5'SS) selection and **exon skipping**. We used a mouse erythroleukemia (MEL) cell line (CB3C7) deficient in the expression of hnRNP **A1** to test whether variations in hnRNP **A1** and

Kam 09/871,809

AlB protein levels affected **alternative splicing** in vivo. In contrast to **A1**-expressing MEL cell lines, CB3C7 cells preferentially selected the proximal 13S and 12S 5'SS on the adenovirus E1A pre-mRNA. Transiently expressing the **A1** or AlB cDNA in CB3C7 cells shifted 5'SS selection toward the more distal 9S donor site. **A1** protein synthesis was required for this effect since the expression of a mutated **A1** cDNA did not affect 5'SS selection. These results demonstrate that in vivo variations in hnRNP **A1** protein levels can influence 5'SS selection.

L29 ANSWER 59 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 94:539069 SCISEARCH
THE GENUINE ARTICLE: PD661
TITLE: SMOOTH MUSCLE-SPECIFIC SWITCHING OF ALPHA-TROPOMYOSIN MUTUALLY EXCLUSIVE EXON SELECTION BY SPECIFIC-INHIBITION OF THE STRONG DEFAULT EXON
AUTHOR: GOODING C; ROBERTS G C; MOREAU G; NADALGINARD B; SMITH C W J (Reprint)
CORPORATE SOURCE: UNIV CAMBRIDGE, DEPT BIOCHEM, TENNIS COURT RD, CAMBRIDGE CB2 1QW, ENGLAND (Reprint); UNIV CAMBRIDGE, DEPT BIOCHEM, CAMBRIDGE CB2 1QW, ENGLAND; HARVARD UNIV, CHILDRENS HOSP, SCH MED, DEPT CELLULAR & MOLEC PHYSIOL, DEPT CARDIOL, BOSTON, MA, 02115
COUNTRY OF AUTHOR: ENGLAND; USA
SOURCE: EMBO JOURNAL, (15 AUG 1994) Vol. 13, No. 16, pp. 3861-3872

ISSN: 0261-4189.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 42
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Exons 2 and 3 of alpha-tropomyosin are spliced in a strict mutually exclusive manner. Exon 3 is a default choice, being selected in almost all cell types where the gene is expressed. The default selection arises from a competition between the two exons, in which the stronger branch point/pyrimidine tract elements of exon 3 win. Exon 2 is selected predominantly or exclusively only in smooth muscle cells. We show here that the basis for the smooth muscle-specific switching of exon selection is inhibition of exon 3. **Exon** 3 is still **skipped** with smooth muscle specificity, even in the absence of exon 2. We have defined two conserved sequence elements, one in each of the introns flanking exon 3, that are essential for this regulation. Mutation of either element severely impairs regulated suppression of exon 3. No other exon or intron sequences appear to be necessary for regulation. We have also demonstrated **skipping** of **exon** 3 that is dependent upon both regulatory elements in an in vitro splicing assay. We further show that both splice sites of exon 3 must be inhibited in a concerted fashion to switch to selection of exon 2. This may relate to the requirement for negative elements on both sides of the exon.

L29 ANSWER 60 OF 73 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 94367370 MEDLINE
DOCUMENT NUMBER: 94367370 PubMed ID: 8085156
TITLE: Regulation of **alternative splicing** in vivo by overexpression of antagonistic splicing factors.
AUTHOR: Caceres J F; Stamm S; Helfman D M; Krainer A R
CORPORATE SOURCE: Cold Spring Harbor Laboratory, NY 11724.
SOURCE: SCIENCE, (1994 Sep 16) 265 (5179) 1706-9.

Search completed by David Schreiber 308-4292

Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941021
Last Updated on STN: 20021218
Entered Medline: 19941012

AB The opposing effects of SF2/ASF and heterogeneous nuclear ribonucleoprotein (hnRNP) **A1** influence **alternative splicing** in vitro. SF2/ASF or hnRNP **A1** complementary DNAs were transiently overexpressed in HeLa cells, and the effect on **alternative splicing** of several cotransfected reporter genes was measured. Increased expression of SF2/ASF activated proximal 5' splice sites, promoted inclusion of a neuron-specific exon, and prevented abnormal **exon skipping**. Increased expression of hnRNP **A1** activated distal 5' splice sites. Therefore, variations in the intracellular levels of antagonistic splicing factors influence different modes of **alternative splicing** in vivo and may be a natural mechanism for tissue-specific or developmental regulation of gene expression.

L29 ANSWER 61 OF 73 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 94088542 MEDLINE
DOCUMENT NUMBER: 94088542 PubMed ID: 8264611
TITLE: Human adenovirus encodes two proteins which have opposite effects on accumulation of **alternatively spliced** mRNAs.
AUTHOR: Nordqvist K; Ohman K; Akusjarvi G
CORPORATE SOURCE: Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institutet, Stockholm, Sweden.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1994 Jan) 14 (1) 437-45.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940209
Last Updated on STN: 19970203
Entered Medline: 19940121

AB All mRNAs expressed from the adenovirus major late transcription unit have a common, 201-nucleotide-long 5' leader sequence, which consists of three short exons (the tripartite leader). This leader has two variants, either with or without the i-leader exon, which, when present, is spliced between the second and the third exons of the tripartite leader. Previous studies have shown that adenovirus early region 4 (**E4**) encodes two proteins, **E4** open reading frame 3 (**E4**-ORF3) and **E4**-ORF6, which are required for efficient expression of mRNAs from the major late transcription unit. These two **E4** proteins appear to have redundant activities, and expression of one has been shown to be sufficient for efficient major late mRNA accumulation during a lytic virus infection. In this report, we provide evidence that **E4**-ORF3 and **E4**-ORF6 both regulate major late mRNA accumulation by stimulating constitutive splicing. Moreover, we show that the two proteins have different effects on accumulation of **alternatively spliced** tripartite leader exons. In a DNA transfection assay, **E4**-ORF3 was shown to facilitate i-leader exon inclusion, while

E4-ORF6 preferentially favored i-leader **exon skipping**. In addition, **E4**-ORF3 and **E4**-ORF6 had the same effects on accumulation of **alternatively spliced** chimeric beta-globin transcripts. This finding suggests that the activities of the two proteins may be of more general relevance and not restricted to splicing of major late tripartite leader-containing pre-mRNAs. Interestingly, **E4**-ORF6 expression was also shown to stimulate i-leader **exon skipping** during a lytic virus infection.

L29 ANSWER 62 OF 73 MEDLINE DUPLICATE 29
ACCESSION NUMBER: 94095205 MEDLINE
DOCUMENT NUMBER: 94095205 PubMed ID: 7505767
TITLE: Exon 9 of the **CFTR** gene: splice site haplotypes and **cystic fibrosis** mutations.
AUTHOR: Dork T; Fislage R; Neumann T; Wulf B; Tummler B
CORPORATE SOURCE: Klinische Forschergruppe Molekulare Pathologie der Mukoviszidose, Zentrum Biochemie, Medizinische Hochschule Hannover, Germany.
SOURCE: HUMAN GENETICS, (1994 Jan) 93 (1) 67-73.
Journal code: 7613873. ISSN: 0340-6717.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940215
Last Updated on STN: 19960129
Entered Medline: 19940203

AB The **alternatively spliced** exon 9 of the **cystic fibrosis** transmembrane conductance regulator (**CFTR**) gene codes for the initial part of the amino-terminal nucleotide-binding fold of **CFTR**. A unique feature of the acceptor splice site preceding this exon is a variable length polymorphism within the polypyrimidine tract influencing the extent of **exon** 9 **skipping** in **CFTR** mRNA. We investigated this repeat for its relationship to **CFTR** mutations and intragenic markers on 200 chromosomes from German patients with **cystic fibrosis** (CF). Four frequent length variations were strongly associated with the four predominant haplotypes previously defined by intragenic marker dimorphisms. One of these alleles displayed absolute linkage disequilibrium to the major CF mutation delta F508. Other frequent **CFTR** mutations were linked to one particular splice site haplotype indicating that differential **exon** 9 **skipping** contributes little to the clinical heterogeneity among CF patients with an identical mutation. We also identified a novel missense mutation (V456F) and a novel nonsense mutation (Q414X) within the coding region of exon 9. The missense mutation V456F adjacent to Walker motif A was present in a pancreas-sufficient CF patient. In contrast, the pancreas-insufficient Q414X/delta F508 compound heterozygote suffered from a severe form of the disease, indicating that **alternative splicing** of exon 9 does not overcome the deleterious effect of a stop codon with this exon.

L29 ANSWER 63 OF 73 MEDLINE DUPLICATE 30
ACCESSION NUMBER: 93233660 MEDLINE
DOCUMENT NUMBER: 93233660 PubMed ID: 8474457
TITLE: Modulation of **exon skipping** and inclusion by heterogeneous nuclear ribonucleoprotein **A1** and pre-mRNA splicing factor SF2/ASF.

Search completed by David Schreiber 308-4292

Kam 09/871,809

COMMENT: Erratum in: Mol Cell Biol 1993 Jul;13(7):4458
AUTHOR: Mayeda A; Helfman D M; Krainer A R
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724-2208.
CONTRACT NUMBER: CA13106 (NCI)
GM43049 (NIGMS)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1993 May) 13 (5) 2993-3001.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930604
Last Updated on STN: 20021218
Entered Medline: 19930518

AB The essential splicing factor SF2/ASF and the heterogeneous nuclear ribonucleoprotein **A1** (hnRNP **A1**) modulate **alternative splicing** in vitro of pre-mRNAs that contain 5' splice sites of comparable strengths competing for a common 3' splice site. Using natural and model pre-mRNAs, we have examined whether the ratio of SF2/ASF to hnRNP **A1** also regulates other modes of **alternative splicing** in vitro. We found that an excess of SF2/ASF effectively prevents inappropriate **exon skipping** and also influences the selection of mutually exclusive tissue-specific exons in natural beta-tropomyosin pre-mRNA. In contrast, an excess of hnRNP **A1** does not cause inappropriate **exon skipping** in natural constitutively or **alternatively spliced** pre-mRNAs. Although hnRNP **A1** can promote alternative **exon skipping**, this effect is not universal and is dependent, e.g., on the size of the internal alternative exon and on the strength of the polypyrimidine tract in the preceding intron. With appropriate alternative exons, an excess of SF2/ASF promotes exon inclusion, whereas an excess of hnRNP **A1** causes **exon skipping**. We propose that in some cases the ratio of SF2/ASF to hnRNP **A1** may play a role in regulating **alternative splicing** by **exon** inclusion or **skipping** through the antagonistic effects of these proteins on **alternative splice** site selection.

L29 ANSWER 64 OF 73 MEDLINE

ACCESSION NUMBER: 94108459 MEDLINE
DOCUMENT NUMBER: 94108459 PubMed ID: 7506605
TITLE: A donor splice mutation (405 + 1 G-->A) in **cystic fibrosis** associated with **exon skipping** in epithelial **CFTR** mRNA.
AUTHOR: Dork T; Will K; Demmer A; Tummler B
CORPORATE SOURCE: Klinische Forschergruppe Molekulare Pathologie der Mukoviszidose, Medizinische Hochschule Hannover, Germany.
SOURCE: HUMAN MOLECULAR GENETICS, (1993 Nov) 2 (11) 1965-6.
Journal code: 9208958. ISSN: 0964-6906.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940228
Last Updated on STN: 19960129
Entered Medline: 19940217

Search completed by David Schreiber 308-4292

Kam 09/871,809

L29 ANSWER 65 OF 73 MEDLINE DUPLICATE 31
ACCESSION NUMBER: 93357737 MEDLINE
DOCUMENT NUMBER: 93357737 PubMed ID: 7689009
TITLE: **Abnormal** mRNA **splicing** resulting from three different mutations in the **CFTR** gene.
AUTHOR: Hull J; Shackleton S; Harris A
CORPORATE SOURCE: Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.
SOURCE: HUMAN MOLECULAR GENETICS, (1993 Jun) 2 (6) 689-92. Journal code: 9208958. ISSN: 0964-6906.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931008
Last Updated on STN: 19960129
Entered Medline: 19930921

AB Three different putative splicing mutations in the **CFTR** gene have been studied by analysing mRNA extracted from nasal epithelial cells harvested from patients with **cystic fibrosis**. Six patients were analysed, all of whom had classical symptoms of **cystic fibrosis** (CF). Two patients carried the 621 + 1G-->T mutation, 3 patients carried the 1717 - 1G-->A mutation and 1 patient carried the 1898 + 1G-->A mutation. All patients carried the delta F508 mutation on the other chromosome. Ten non-CF control subjects were also studied. The 621 + 1G-->T mutation resulted in activation of an **alternative splice** site within exon 4 in one patient and activation of this site or **skipping** of **exon** 4 in the other patient. The 1717 - 1G-->A mutation resulted in **skipping** of **exon** 11 in all 3 patients studied and the 1898 + 1G-->T mutation resulted in **skipping** of **exon** 12. These experiments demonstrate that these mutations do result in **aberrant splicing** of **CFTR** mRNA as predicted from the changes in genomic sequence.

L29 ANSWER 66 OF 73 MEDLINE
ACCESSION NUMBER: 94004974 MEDLINE
DOCUMENT NUMBER: 94004974 PubMed ID: 7691356
TITLE: **Cystic fibrosis** transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels.
AUTHOR: Delaney S J; Rich D P; Thomson S A; Hargrave M R; Lovelock P K; Welsh M J; Wainwright B J
CORPORATE SOURCE: Centre for Molecular Biology and Biotechnology, University of Queensland, Brisbane, Australia.
SOURCE: NATURE GENETICS, (1993 Aug) 4 (4) 426-31. Journal code: 9216904. ISSN: 1061-4036.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19960129
Entered Medline: 19931105

AB In the human **CFTR** only the rare exon 4- splice variant is conserved in mice. We have discovered two novel murine variants, exon 5-

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and exon 11b+. The exon 5- variant represents up to 40% of mRNA in all **CFTR**-expressing tissues and leaves the reading frame intact. The exon 11b+ variant inserts a novel exon between exons 11 and 12 with expression restricted to the testis. Two variants of 11b have been found and both introduce premature stop codons. When we expressed human **CFTR** variants lacking either exon 5 or exon 9 in HeLa cells, they failed to generate cAMP-mediated chloride transport, due to defective intracellular processing. The lack of conservation of splice variants between species and the inability of the more abundant splice variants to generate protein that is correctly processed argue against a physiological role and may simply represent **aberrant splicing** that is tolerated by the cell and organism.

L29 ANSWER 67 OF 73 MEDLINE
ACCESSION NUMBER: 95072652 MEDLINE
DOCUMENT NUMBER: 95072652 PubMed ID: 7981749
TITLE: A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells.
AUTHOR: Matsuo T; Osumi-Yamashita N; Noji S; Ohuchi H; Koyama E; Myokai F; Matsuo N; Taniguchi S; Doi H; Iseki S; +
CORPORATE SOURCE: Department of Ophthalmology, Okayama University Medical School, Japan.
SOURCE: NATURE GENETICS, (1993 Apr) 3 (4) 299-304.
Journal code: 9216904. ISSN: 1061-4036.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950116
Last Updated on STN: 19950116
Entered Medline: 19950105

AB The rat small eye strain (rSey) lacks eyes and nose in the homozygote, and is similar to the mouse Sey strain with mutations in the Pax-6 gene. We isolated Pax-6 cDNA clones from an rSey homozygote library, and found an internal deletion of about 600 basepairs in the **serine**/threonine-**rich** domain. At the genomic level, a single base (G) insertion in an exon generates an **abnormal** 5' donor **splice** site, thereby producing the truncated mRNA. Anterior midbrain crest cells in the homozygous rSey embryos reached the eye rudiments but did not migrate any further to the nasal rudiments, suggesting that the Pax-6 gene is involved in conducting migration of neural crest cells from the anterior midbrain.

L29 ANSWER 68 OF 73 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93079798 EMBASE
DOCUMENT NUMBER: 1993079798
TITLE: Genetic basis of variable **exon** 9 **skipping** in **cystic fibrosis** transmembrane conductance regulator mRNA.
AUTHOR: Chu C.-S.; Trapnell B.C.; Curristin S.; Cutting G.R.; Crystal R.G.
CORPORATE SOURCE: Pulmonary Branch, National Heart Lung and Blood Inst, National Institutes of Health,Bethesda, MD 20892, United States
SOURCE: Nature Genetics, (1993) 3/2 (151-156).
ISSN: 1061-4036 CODEN: NGENEC
COUNTRY: United States
DOCUMENT TYPE: Journal; Article

Search completed by David Schreiber 308-4292

Kam 09/871,809

FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Variable in-frame **skipping** of **exon** 9 in **cystic fibrosis** transmembrane conductance regulator (**CFTR**) mRNA transcripts (exon 9-) occurs in the respiratory epithelium. To explore the genetic basis of this event, we evaluated respiratory epithelial cells and blood leukocytes from 124 individuals (38 with **cystic fibrosis** (CF), 86 without CF). We found an inverse relationship between the length of the polythymidine tract at the exon 9 splice branch/acceptor site and the proportion of exon 9- **CFTR** mRNA transcripts. These results strongly indicate a genetic basis in vivo modulating post-transcriptional processing of **CFTR** mRNA transcripts.

L29 ANSWER 69 OF 73 MEDLINE DUPLICATE 32
ACCESSION NUMBER: 94040719 MEDLINE
DOCUMENT NUMBER: 94040719 PubMed ID: 1285125
TITLE: U1 snRNP targets an essential splicing factor, U2AF65, to the 3' splice site by a network of interactions spanning the exon.
AUTHOR: Hoffman B E; Grabowski P J
CORPORATE SOURCE: Department of Biological Sciences, University of Pittsburgh, Pennsylvania 15260.
CONTRACT NUMBER: GM39695 (NIGMS)
SOURCE: GENES AND DEVELOPMENT, (1992 Dec) 6 (12B) 2554-68. Journal code: 8711660. ISSN: 0890-9369.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 20000303
Entered Medline: 19931201
AB A description of cellular factors that govern **alternative splicing** of pre-mRNA is largely incomplete. In the case of the rat preprotachykinin gene, **splicing** of the **alternative** exon **E4** occurs by a poorly understood mechanism in which exon selection is under the positive control of U1 snRNP. Because the binding of U1 snRNP to the 5' splice site of **E4** is coincident with the selection of the 3' splice site of **E4**, this mechanism would appear to involve interactions that bridge across the exon. In this work, a UV cross-linking strategy was used to identify possible RNA-protein interactions involved in the proposed exon-bridging model. Of particular interest is a prominent 61-kD protein, p61, that binds to the 3' splice site of **E4** in a manner that is clearly facilitated by a downstream 5' splice site and U1 snRNP particles. The identity of p61 is the essential splicing factor U2AF65, on the basis of copurification and selective binding to polypyrimidine tracts. These results indicate a model in which exon selection is positively regulated by the communication of U1 snRNP and U2AF65. That is, a natural deficiency in binding U2AF65 to the 3' splice site that leads to **exon skipping** might be overcome by a mechanism in which U1 snRNP facilitates the binding of U2AF65 through a network of template-directed and exon-bridging interactions.

L29 ANSWER 70 OF 73 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92203300 EMBASE

Search completed by David Schreiber 308-4292

Kam 09/871,809

DOCUMENT NUMBER: 1992203300
TITLE: Activation of c-**src** neuron-specific splicing by an unusual RNA element in vivo and in vitro.
AUTHOR: Black D.L.
CORPORATE SOURCE: Nine Cambridge Center, Whitehead Biomedical Research Inst.,Cambridge, MA 02142, United States
SOURCE: Cell, (1992) 69/5 (795-807).
ISSN: 0092-8674 CODEN: CELLB5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A conserved positive-acting RNA sequence was found to be required for the neuron-specific splicing of the mouse c-**src** N1 exon. The sequence lies in the intron between exons N1 and 4, close to the N1 donor site. **Normally**, only the neural-specific splicing of exon N1 required this sequence. When the intron downstream of N1 was shortened, splicing at the constitutive exon 4 acceptor also became dependent on the activating sequence. The neuronal and nonneuronal patterns of **src** splicing were reconstituted in vitro. HeLa cell extracts spliced exon 4 to **exon** 3, **skipping exon** N1. Weri-1 retinoblastoma cell extracts spliced exon 4 to exon N1 as well as to exon 3. Both patterns of splicing were dependent on the activating sequence. A 123 nt RNA containing just the activating sequence specifically inhibited both patterns of **src** splicing, indicating that factors bound to the activator were required for its effects.

L29 ANSWER 71 OF 73 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 91:147968 SCISEARCH
THE GENUINE ARTICLE: FB280
TITLE: DOES STERIC INTERFERENCE BETWEEN SPLICE SITES BLOCK THE SPLICING OF A SHORT C-**SRC** NEURON-SPECIFIC EXON IN NONNEURONAL CELLS
AUTHOR: BLACK D L (Reprint)
CORPORATE SOURCE: WHITEHEAD INST BIOMED RES, CAMBRIDGE, MA, 02142 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: GENES & DEVELOPMENT, (1991) Vol. 5, No. 3, pp. 389-402.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 77

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The neuron-specific splicing of the mouse c-**src** N1 exon was analyzed. Model **src** genes, transiently expressed in HeLa and LA-N-5 neuroblastoma cells, were assayed for the insertion of the 18-nucleotide neuron-specific N1 exon into their product mRNA. The **normal** clone fails to use this exon in HeLa cells but inserts the exon into 50% of the mature mRNA in LA-N-5 cells. When the exon and flanking intron sequences are placed between two adenovirus exons, the N1 exon is still only inserted in the neural cells. Thus, the neural specificity is a property of the exon itself and its immediate flanking sequences. Simply extending the length of the N1 exon to 109 nucleotides allows its efficient use in HeLa cells, implying that the **exon** is **normally skipped** because it is too short to allow spliceosomes to assemble at both ends simultaneously. This model predicts that exclusion of the exon should be sensitive to proteins or mutations

Search completed by David Schreiber 308-4292

that alter the relative strength of the flanking splice sites. Mutations that change these splice sites support this hypothesis.

L29 ANSWER 72 OF 73 MEDLINE DUPLICATE 33
ACCESSION NUMBER: 91309150 MEDLINE
DOCUMENT NUMBER: 91309150 PubMed ID: 1830244
TITLE: Functional expression of cloned human splicing factor SF2: homology to RNA-binding proteins, U1 70K, and Drosophila splicing regulators.
AUTHOR: Krainer A R; Mayeda A; Kozak D; Binns G
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724-2208.
CONTRACT NUMBER: CA13106 (NCI)
GM42699 (NIGMS)
SOURCE: CELL, (1991 Jul 26) 66 (2) 383-94.
Journal code: 0413066. ISSN: 0092-8674.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M69039; GENBANK-M69040
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910913
Last Updated on STN: 19970203
Entered Medline: 19910827

AB SF2 is a protein factor essential for constitutive pre-mRNA splicing in HeLa cell extracts and also activates proximal **alternative** 5' **splice** sites in a concentration-dependent manner. This latter property suggests a role for SF2 in preventing **exon skipping**, ensuring the accuracy of **splicing**, and regulating **alternative splicing**. Human SF2 cDNAs have been isolated and overexpressed in bacteria. Recombinant SF2 is active in splicing and stimulates proximal 5' splice sites. SF2 has a C-terminal region **rich** in **arginine-serine** dipeptides, similar to the RS domains of the U1 snRNP 70K polypeptide and the Drosophila **alternative splicing** regulators transformer, transformer-2, and suppressor-of-white-apricot. Like transformer-2 and 70K, SF2 contains an RNP-type RNA recognition motif.

L29 ANSWER 73 OF 73 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:93122 HCAPLUS
DOCUMENT NUMBER: 112:93122
TITLE: An intron mutation in the human .alpha.1(I) collagen gene alters the efficiency of pre-mRNA splicing and is associated with osteogenesis imperfecta type II
AUTHOR(S): Bonadio, Jeffrey; Ramirez, Francesco; Barr, Mason
CORPORATE SOURCE: Howard Hughes Med. Inst., Univ. Michigan, Ann Arbor, MI, 48109-0650, USA
SOURCE: Journal of Biological Chemistry (1990), 265(4), 2262-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This study describes a homozygous, G .fwdarw. A transition at the moderately conserved +5 position within the splice donor site of intron 14 in the human .alpha.1 (I) collagen gene. The mutation reduced the efficiency of **normal** splice-site selection since the exon upstream of the mutation was **spliced alternatively**. Moreover, the extent of **alternative splicing** was sensitive to the temp. at which the mutant cells were grown, suggesting that the mutation directly affected spliceosome assembly. To achieve

exon skipping, this effect must be propagated so as to disrupt the selection of a second splice site in the adjacent intron.

```
=> d que l30
L1          347 SEA KEREM B?/AU
L2            2 SEA L1 AND ABERRANT(A) SPLIC?
L3         3926 SEA (ABERRANT OR ABNORMAL)(3A) SPLIC?
L4         4966 SEA EXON(3A) SKIP?
L5          175 SEA INTRON(3A) EXCLU?
L6        58172 SEA ALTERNATIV?(3A) SPLIC?
L7          488 SEA ERRO?(5A) SPLIC?
L8         8805 SEA (L3 OR L4 OR L5) OR L7
L9         1977 SEA L8 AND L6
L11          23 SEA L9 AND E4?
L12          70 SEA L9 AND A1
L18         229 SEA (L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17)
L19        6768 SEA TRANSITION(3A) MUTA?
L20          12 SEA L9 AND L19
L21         240 SEA L18 OR L20
L22        5628 SEA C(3A) FWDARW(3A) T
L23          10 SEA L22 AND L9
L24         246 SEA L21 OR L23
L25          30 SEA L24 AND NORMAL?
L26          24 SEA L24 AND (TREAT? OR THERAP? OR ADMINIST?)
L27         158 SEA L24 NOT PY>1999
L28         187 SEA L27 OR L25 OR L26
L29          73 DUP REM L28 (114 DUPLICATES REMOVED)
L30           1 SEA L2 NOT L29

=> d ibib abs l30 1

L30  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:        1997:59673  HCAPLUS
DOCUMENT NUMBER:         126:87931
TITLE:                   The molecular basis of partial penetrance of splicing
                         mutations in cystic fibrosis
AUTHOR(S):               Rave-Harel, Maama; Kerem, Eitan; Nissim-Rafinia,
                         Malka; Madjar, Igal; Goshen, Ran; Augarten, Arie;
                         Rahat, Avelet; Hurwitz, Arye; Darvasi, Ariel;
                         Kerem, Batsheva
CORPORATE SOURCE:        Life Sciences Institute, Hebrew University, Jerusalem,
                         Israel
SOURCE:                  American Journal of Human Genetics (1997), 60(1),
                         87-94
                         CODEN: AJHGAG; ISSN: 0002-9297
PUBLISHER:               University of Chicago Press
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   The splicing variant, 5T allele, in intron 8 of the cystic fibrosis
     transmembrane conductance regulator (CFTR) gene was shown to be assocd.
     with partial penetrance of the clin. expression.  This splicing variant
     leads to two possible transcripts: one normal and the other aberrantly
     spliced that lacks exon 9.  The aim of this study was to analyze the mol.
     basis of the partial penetrance in individuals carrying the 5T allele.
     The authors analyzed the level of the correctly spliced RNA transcribed
     from the 5T allele in nasal and epididymal epithelium and correlated it
     with disease expression.  Semiquant. nondifferential reverse-transcriptase-
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PCR showed a considerable variability (6%-37%) in the total level of correctly spliced RNA transcribed from the 5T allele in nasal epithelium from 11 patients. A significant nonlinear correlation (r =.82) between the level of the normal CFTR transcripts and the severity of lung disease was shown. No individuals with normal lung function and minimal or no lung disease (FEV1 >80% predicted) had <25% of normal transcripts, and individuals with <15% of normal transcripts did not have FEV1 >80%. The level of normal transcripts in epididymal epithelial cells from four infertile males with congenital bilateral absence of the vas deferens was low (6-24%). In infertile males with normal lung function the level of correctly spliced transcripts in the nasal epithelium was higher than the level in the epididymal epithelium. These results indicate that there is variability in the efficiency of the splicing mechanism, among different individuals and between different organs of the same individual. This variability provides the mol. basis of the partial penetrance of cystic fibrosis disease in patients carrying the 5T allele.

Age Break
Page Numbering
Application #

09/421,891

10(b) 10/20/98
10(a) 10/21/98
10/20/99

L12 ANSWER 1 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 2000011380 EMBASE
TITLE: Differential expression of the splicing regulatory factor genes during two-step chemical transformation in a BALB/3T3-derived cell line, MT-5.
AUTHOR: Maeda T.; Hiranuma H.; Jikko A.
CORPORATE SOURCE: T. Maeda, Dept. Radiology Radiation Oncology, Osaka University, School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan. tmaeda@radiol.dent.osaka-u.ac.jp
SOURCE: Carcinogenesis, (1999) 20/12 (2341-2344).
Refs: 24
ISSN: 0143-3334 CODEN: CRNGDP
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Although the alternative splicing of various genes is a common event in human tumors, the mechanisms behind it have not been characterized. We hypothesized that the expression of splicing regulatory factors would be changed during cellular transformation. **Gene** expression of three splicing regulatory factors, **alternative splicing factor/splicing factor** 2 (ASF/SF2), heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2) and the 65 kDa subunit of U2 small nuclear ribonucleoprotein particles auxiliary factor (U2AF65), were examined by northern blotting in a two-step chemical transformation model. This in vitro model is composed of BALB/3T3 cells and a BALB/3T3-derived N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-initiated cell line (MT-5). MT-5 cells can be transformed on exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA). ASF/SF2 mRNA levels were decreased 2-fold in both MNNG-initiated cells and TPA-induced transformed cells compared with the normal parental cells, whereas hnRNP A2 mRNA expression did not significantly change between these three types of cells. U2AF65 mRNA levels were markedly increased (.apprx. 4.7-fold) associated with progression of cellular transformation. Moreover, RT-PCR analysis showed that distinct forms of ASF/SF2 mRNA were present in the MNNG-initiated cells and TPA-induced transformed cells but not in the parental cells. These findings indicate that ASF/SF2 or U2AF65 **gene** expression is altered during in vitro two-step chemical transformation. The data suggest that the differential expression of [illegible] is the cause of aberrant expression of alternatively spliced mRNAs encoded by various genes in tumor cells.

L12 ANSWER 2 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 1999418938 EMBASE
TITLE: Functional analysis of cis-acting elements regulating the alternative splicing of human CFTR exon 9.
AUTHOR: Niksic M.; Romano M.; Buratti E.; Pagani F.; Baralle F.E.
CORPORATE SOURCE: F.E. Baralle, ICGEB, Padriciano 99, 34012 Trieste, Italy. baralle@icgeb.trieste.it
SOURCE: Human Molecular Genetics, (1999) 8/13 (2339-2349).
Refs: 40
ISSN: 0964-6906 CODEN: HMGEE5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The rate of exon 9 exclusion from the **cystic fibrosis** transmembrane conductance regulator (CFTR) mRNA is associated with monosymptomatic forms of **cystic fibrosis**. Exon 9 **alternative splicing** is modulated by a polymorphic polythymidine tract within its 3' splice site. We have generated a minigene carrying human CFTR exon 9 with its flanking intronic sequences and set up an in vivo model to study the cis-acting DNA elements which modulate its splicing. Transfections into human cell lines showed that T5, but not T9 or T7 alleles, significantly increases the **alternative splicing** of exon 9. Moreover, we found that another polymorphic locus juxtaposed upstream of the T tract, and constituted by (TG)(n) repeats, can further modulate exon 9 skipping but only when activated by the T5 allele. Then, we extended our studies to the mouse CFTR exon 9 which does not show **alternative splicing**. Comparison of human and mouse introns 8 and 9 revealed a low homology between the two sequences and the absence of the human polymorphic loci within the mouse intron 3' splice site. We have tested a series of constructs where the whole human exon 9 with its flanking intronic sequences was replaced partially or completely by the murine counterpart. The transfections of these constructs in human and murine cell lines reveal that also sequences of the downstream intron 9 affect exon 9 definition and co-modulate, with the UG/U 3' splice site sequences, the extent of exon 9 skipping in CFTR mRNA.

L12 ANSWER 3 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3
ACCESSION NUMBER: 1999208100 EMBASE
TITLE: Variable levels of normal RNA in different fetal organs carrying a cystic fibrosis transmembrane conductance regulator splicing mutation.
AUTHOR: Chiba-Falek O.; Parad R.B.; Kerem E.; Kerem B.
CORPORATE SOURCE: Dr. B. Kerem, Department of Genetics, Life Sciences Institute, Hebrew University, Jerusalem 91904, Israel. kerem@.leonardo.ls.huji.ac.il
SOURCE: American Journal of Respiratory and Critical Care Medicine, (1999) 159/6 (1998-2002).
Refs: 19
ISSN: 1073-449X CODEN: AJCMED
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Disease severity varies among **cystic fibrosis** (CF) patients carrying the same **cystic fibrosis** transmembrane conductance regulator (CFTR) genotype and among organs of the same individual. It has been shown that the class V splicing mutation 3849 + 10 kb C .fwdarw. T produces both normal and aberrantly spliced CFTR transcripts. We analyzed the levels of normal CFTR messenger RNA (mRNA) in different organs of an aborted fetus carrying the 3849 + 10 kb C .fwdarw. T mutation, and found that they correlated with the histopathologic changes observed in these organs. We performed semiquantitative nondifferential reverse transcription-polymerase chain reaction on several organs from a 22- wk aborted CF fetus carrying the 3849 + 10 kb C .fwdarw. T mutation. A very low level (1%) of normal CFTR mRNA was detected in the severely affected ileum of this fetus. Higher levels were found in the histopathologically unaffected trachea (17%), colon (19%), and lung (26%).

Thus, as early as in utero, the regulation of alternative splice-site selection is an important mechanism underlying variable CF severity. Understanding of the mechanisms regulating **alternative splicing** in different tissues will contribute to potential therapy for patients carrying splicing mutations in CF and other human disease genes.

L12 ANSWER 4 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 4
ACCESSION NUMBER: 1999036401 EMBASE
TITLE: Dual posttranscriptional targets of retinoic acid-induced **gene** expression.
AUTHOR: Manji S.S.; Pearson R.B.; Pardee M.; Paspaliaris V.; D'Apice A.; Martin T.J.; Kong Wah Ng
CORPORATE SOURCE: K.W. Ng, Department of Medicine, University of Melbourne, St. Vincent's Hospital, Fitzroy, Vic. 3065, Australia. k.ng@medicine.unimelb.edu.au
SOURCE: Journal of Cellular Biochemistry, (1 Mar 1999) 72/3 (411-422).
Refs: 45
ISSN: 0730-2312 CODEN: JCEBD5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Retinoic acid-induced differentiation of the pre-osteoblastic cell line, UMR 201, is associated with a marked increase in the proficiency of posttranscriptional nuclear processing of alkaline phosphatase mRNA. In this study we attempted to correlate the posttranscriptional actions of retinoic acid with changes in phosphorylation, or abundance of spliceosome components, or both. Treatment with retinoic acid for periods of .ltoreq.4 h resulted in dephosphorylation of nuclear U1 70K protein without affecting its abundance. Peptide mapping showed that U1 70K dephosphorylation was related to the disappearance of one specific phosphopeptide out of four major U1 70K phosphopeptides. A twofold decrease in mRNA expression of an isoform of **alternative splicing factor** that inhibits splicing was also observed over the same period. Tumor necrosis factor-.alpha., which enhances the posttranscriptional action of retinoic acid, reduced U170K mRNA expression, while an inhibition of retinoic acid action by transforming growth factor-.beta. was associated with a marked increase in U1 70K mRNA levels. Our results draw attention to the complex interactions between short- and long-term alterations in the abundance and functional status of [illegible] as well as SR proteins by growth and/or differentiation factors in the regulation of spliceosome formation and function.

L12 ANSWER 5 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 5
ACCESSION NUMBER: 1999049258 EMBASE
TITLE: Oxidative stress induces differential **gene** expression in a human lens epithelial cell line.
AUTHOR: Carper D.A.; Sun J.K.; Iwata T.; Zigler J.S. Jr.; Ibaraki N.; Lin L.-R.; Reddy V.
CORPORATE SOURCE: D.A. Carper, Building 6, National Eye Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, United States
SOURCE: Investigative Ophthalmology and Visual Science, (1999) 40/2
(400-406).
Refs: 37
ISSN: 0146-0404 CODEN: IOVSDA

COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
012 Ophthalmology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB PURPOSE. To identify differentially expressed genes in a human lens epithelial cell line exposed to oxidative stress. METHODS. Reverse transcriptase-polymerase chain reaction (RT-PCR) differential display was used to evaluate differential **gene** expression in a human lens epithelial cell line (SRA 01-04) when cells were exposed for 3 hours to a single bolus of 200 .mu.M hydrogen peroxide. Differentially expressed genes were identified through DNA sequencing and a nucleotide database search. Differential expression was confirmed by northern blot and RT-PCR analyses. RESULTS. Using 18 primer sets, 28 RT-PCR products were differentially expressed between control and hydrogen peroxide-treated cells. In stressed cells, mitochondrial transcripts nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 4 and cytochrome b were downregulated 4-fold. Of the cytoplasmic mRNAs, glutamine cyclotransferase decreased 10-fold, whereas cytokine-inducible nuclear protein, **alternative splicing factor** 2, and .beta.-hydroxyisobutyryl-coenzyme A hydrolase increased 2-, 4-, and 10-fold, respectively. Analysis of mitochondrial transcripts in a 24-hour time course showed that NADH dehydrogenase subunit 4 mRNA decreased by 2-fold as early as 1 hour after oxidative stress, whereas the rate of decrease was slower for cytochrome b, cytochrome oxidase III, and 16S rRNA. CONCLUSIONS. Oxidative stress induced specific expressed **gene** changes in hydrogen peroxide-treated lens cells, including genes involved in cellular respiration and mRNA and peptide processing. These early changes may reflect pathways involved in the defense, pathology, or both of the lens epithelium, which is exposed to oxidative stress throughout life.

L12 ANSWER 6 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 6
ACCESSION NUMBER: 1999277803 EMBASE
TITLE: Cystic fibrosis patients with the 3272-26A.fwdarw.G mutation have mild disease, leaky alternative mRNA splicing, and CFTR protein at the cell membrane.
AUTHOR: Beck S.; Penque D.; Garcia S.; Gomes A.; Farinha C.; Mata L.; Gulbenkian S.; Gil-Ferreira K.; Duarte A.; Pacheco P.; Barreto C.; Lopes B.; Cavaco J.; Lavinha J.; Amaral M.D.
CORPORATE SOURCE: M.D. Amaral, Centro de Genetica Humana, Instituto Nacional de Saude, Av. Padre Cruz, 1649-016 Lisboa, Portugal. mbotelho@pen.gulbenkian.pt
SOURCE: Human Mutation, (1999) 14/2 (133-144).
Refs: 35
ISSN: 1059-7794 CODEN: HUMUE3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 011 Otorhinolaryngology
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We characterized the 3272-26A.fwdarw.G mutation in the **cystic fibrosis** transmembrane conductance regulator (CFTR) gene, creating an alternative acceptor splice site in intron 17a, that competes with the normal one, although we predict from consensus values, with lower efficiency. We analyzed five **Cystic Fibrosis** (CF) Portuguese patients with the 3272-26A.fwdarw.G/F508del genotype. Besides clinical and haplotype characterization of those patients, we report here results from CFTR transcript analysis in nasal brushings from all five patients. RT-PCR analysis supports **alternative splicing** in all patients and carriers, but not in controls. By sequencing, we

determined that the alternative transcript includes 25 nucleotides from intron 17a, which predictively cause frameshift and a premature stop codon. The use of this alternative splice site causes a reduction in the levels of normal transcripts from the allele with this mutation and, most probably, of normal protein as well. By immunocytochemistry of both epithelial primary cell cultures and slices from CF polyps, CFTR protein is detected at the cell membrane, with three different antibodies. Using chamber analysis of one nasal polyp shows a high sodium absorption, characteristic of CF. Altogether, the results suggest that the main defect caused by the 3272-26A.fwdarw.G mutation is a reduction in normal CFTR transcripts and protein and therefore this mutation should be included in class V, according to Zielenski and Tsui.

L12 ANSWER 7 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998409483 EMBASE
TITLE: A pyrimidine-rich exonic splicing suppressor binds multiple RNA splicing factors and inhibits spliceosome assembly.
AUTHOR: Zheng Z.-M.; Huynen M.; Baker C.C.
CORPORATE SOURCE: Z.-M. Zheng, Basic Research Laboratory, Division of Basic Sciences, National Cancer Institute, Bethesda, MD 20892-5055, United States. zhengt@dce41.nci.nih.gov
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (24 Nov 1998) 95/24 (14088-14093).
Refs: 49
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The bovine papillomavirus type 1 (BPV-1) exonic splicing suppressor (ESS) is juxtaposed immediately downstream of BPV-1 splicing enhancer 1 and negatively modulates selection of a suboptimal 3' splice site at nucleotide 3225. The present study demonstrates that this pyrimidine-rich ESS inhibits utilization of upstream 3' splice sites by blocking early steps in spliceosome assembly. Analysis of the proteins that bind to the ESS showed that the U-rich 5' region binds U2AF65 and polypyrimidine tract binding protein, the C-rich central part binds 35- and 54-55-kDa serine/arginine- rich (SR) proteins, and the AG-rich 3' end binds **alternative splicing factor/splicing factor** 2. Mutational and functional studies indicated that the most critical region of the ESS maps to the central C-rich core (GGCUCCCCC). This core sequence, along with additional nonspecific downstream nucleotides, is sufficient for partial suppression of spliceosome assembly and splicing of BPV-1 pre-mRNAs. The inhibition of splicing by the ESS can be partially relieved by excess purified HeLa SR proteins, suggesting that the ESS suppresses pre-mRNA splicing by interfering with normal bridging and recruitment activities of SR proteins.

L12 ANSWER 8 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 7
ACCESSION NUMBER: 1998341611 EMBASE
TITLE: Testicular CFTR splice variants in patients with congenital absence of the vas deferens.
AUTHOR: Larriba S.; Bassas L.; Gimenez J.; Ramos M.D.; Segura A.; Nunes V.; Estivill X.; Casals T.
CORPORATE SOURCE: T. Casals, Medical Molecular Genetics Ctr.-IRO, Hospital Duran i Reynals, Hospitalet Llobregat 08907 Barcelona, Spain. tcasals@iro.es
SOURCE: Human Molecular Genetics, (1998) 7/11 (1739-1744).

Refs: 38
ISSN: 0964-6906 CODEN: HMGEE5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
028 Urology and Nephrology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The involvement of the five thymidine (5T) variant in intron 8 of the **cystic fibrosis** membrane regulator (CFTR) gene in congenital bilateral absence of the vas deferens (CBAVD) phenotype has been extensively demonstrated. This variant leads to **alternative splicing** of the CFTR gene which results in a wild-type transcript and one without exon 9. Little is known about expression of the CFTR gene in the testis. We analysed the level of the aberrantly spliced transcripts in testicular biopsies and correlated it with disease expression. Quantitative RT-PCR analysis in testicular biopsies from control and CBAVD patients showed a correlation between the length of the IVS8-6(T)(n) tract and the level of alternatively spliced transcripts. Results from histological analysis also suggest an involvement of the alternative transcript in the spermatogenic status of patients, leading to a decreased number of mature sperm forms in the tubule.

5T + Cystic Fibrosis

L12 ANSWER 9 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 8
ACCESSION NUMBER: 1998028062 EMBASE
TITLE: Development of an epithelium-specific expression cassette with human DNA regulatory elements for transgene expression in lung airways.
AUTHOR: Chow Y.-H.; O'Brodovich H.; Plumb J.; Wen Y.; Sohn K.-J.; Lu Z.; Zhang F.; Lukacs G.L.; Tanswell A.K.; Hui C.-C.; Buchwald M.; Hu J.
CORPORATE SOURCE: Y.-H. Chow, Division of Respiratory Research, University of Toronton, Toronto, Ont. M5G 1X8, Canada
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1997) 94/26 (14695-14700).
Refs: 28
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The efficient expression of therapeutic genes in target cells or tissues in an important component of efficient and safe gene therapy. Utilizing regulatory elements from the human cytokeratin 18 (K18) gene, including 5' genomic sequences and one of its introns, we have developed a novel expression cassette that can efficiently express reporter genes, as well as the human **cystic fibrosis** transmembrane conductance regulator (CFTR) gene, in cultured lung epithelial cells. CFTR transcripts expressed from the native K18 enhancer/promoter include two **alternative splicing** products, due to the activation of two cryptic splice sites in the CFTR coding region. Modification of the K18 intro and CFTR cDNA sequences eliminated the cryptic splice sites without changing the CFTR amino acid sequence, and led to enhanced CFTR mRNA and protein expression as well as biological function. Transgenic expression analysis in mice showed that the modified expression cassette can direct efficient and epithelium-specific expression of the Escherichia

coli LacZ gene in the airways of fetal lungs, with no detectable expression lung fibroblasts or endothelial cells. This is the first expression cassette which selectively directs lung transgene expression for CFTR gene therapy to airway epithelia.

L12 ANSWER 10 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 9
ACCESSION NUMBER: 97344242 EMBASE
DOCUMENT NUMBER: 1997344242
TITLE: Higher proportion of intact exon 9 CFTR mRNA in nasal epithelium compared with vas deferens.
AUTHOR: Mak V.; Jarvi K.A.; Zielenski J.; Durie P.; Tsui L.-C.
CORPORATE SOURCE: L.-C. Tsui, Department of Genetics, The Hospital for Sick Children, Toronto, Ont. M5G 1X8, Canada. lctsui@genet.sickkids.on.ca
SOURCE: Human Molecular Genetics, (1997) 6/12 (2099-2107). Refs: 47 ISSN: 0964-6906 CODEN: HMGEE5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 010 Obstetrics and Gynecology
022 Human Genetics
028 Urology and Nephrology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The 5-thymidine (5T) variant of the **cystic fibrosis** transmembrane conductance regulator (CFTR) intron 8 polypyrimidine tract (IVS8-T tract) is the most frequent CFTR gene alteration identified in men with congenital bilateral absence of vas deferens (CBAVD). This **alternative splicing** variant gives rise to two transcripts, one normal with exon 9 intact and the other with in-frame deletion of exon 9. That CBAVD men usually have none of the other clinical signs of classical **cystic fibrosis** (CF) suggests less functional CFTR is produced in the reproductive tract than in other CF-associated organs. Nasal epithelia and segments of vas deferens were obtained from healthy, previously vasectomized men who presented for vasectomy reversal. Quantitative RT-PCR was performed on these specimens, with the region of CFTR cDNA spanning exon 9 amplified. For both nasal and vasal tissues, a strong positive correlation was found between the length of the IVS8-T tract and the proportion of mRNA with exon 9 intact. In addition, within the same subject, a significantly higher level of transcripts lacking exon 9 was found in vas deferens than nasal epithelia, regardless of the IVS8-T genotype. These findings suggest that the splicing of CFTR precursor mRNA is less efficient in vasal epithelia compared with respiratory epithelia. Thus, differential splicing efficiency between the various tissues which express CFTR provides one possible explanation for the reproductive tract abnormalities observed in infertile men with CFTR gene alterations but without other clinical manifestations of CF.

L12 ANSWER 11 OF 45 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:274027 CAPLUS
DOCUMENT NUMBER: 126:313674
TITLE: The SR splicing factors ASF/SF2 and SC35 have antagonistic effects on intronic enhancer-dependent splicing of the .beta.-tropomyosin alternative exon 6A
AUTHOR(S): Gallego, Maria E.; Gattoni, Renata; Stevenin, James; Marie, Joelle; Expert-Bezancon, Alain
CORPORATE SOURCE: Cent. Genetique Moleculaire, Cent. Natl. Recherche Sci., Gif-sur-Yvette, 91190, Fr.
SOURCE: EMBO J. (1997), 16(7), 1772-1784

Electronic

CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Exons 6A and 6B of the chicken .beta.-tropomyosin **gene** are mutually exclusive and selected in a tissue-specific manner. Exon 6A is present in non-muscle and smooth muscle cells, while exon 6B is present in skeletal muscle cells. In this study we have investigated the mechanism underlying exon 6A recognition in non-muscle cells. Previous reports have identified a pyrimidine-rich intronic enhancer sequence (S4) downstream of exon 6A as essential for exon 6A 5'-splice site recognition. We show here that preincubation of HeLa cell exts. with an excess of RNA contg. this sequence specifically inhibits exon 6A recognition by the splicing machinery. Splicing inhibition by an excess of this RNA can be rescued by addn. of the SR protein ASF/SF2, but not by the SR proteins SC35 or 9G8. ASF/SF2 stimulates exon 6A splicing through specific interaction with the enhancer sequence. Surprisingly, SC35 behaves as an inhibitor of exon 6A splicing, since addn. to HeLa nuclear exts. of increasing amts. of the SC35 protein completely abolish the stimulatory effect of ASF/SF2 on exon 6A splicing. We conclude that exon 6A recognition in vitro depends on the ratio of the ASF/SF2 to SC35 SR proteins. Taken together our results suggest that variations in the level or activity of these proteins could contribute to the tissue-specific choice of .beta.-tropomyosin exon 6A. In support of this we show that SR proteins isolated from skeletal muscle tissues are less efficient for exon 6A stimulation than SR proteins isolated from HeLa cells.

SR Proteins
ASF/SF2

L12 ANSWER 12 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 10
ACCESSION NUMBER: 97336773 EMBASE
DOCUMENT NUMBER: 1997336773
TITLE: Analysis of the intron-exon organization of the human multidrug- resistance protein gene (MRP) and alternative splicing of its mRNA.
AUTHOR: Grant C.E.; Kurz E.U.; Cole S.P.C.; Deeley R.G.
CORPORATE SOURCE: R.G. Deeley, Cancer Research Laboratories, Queen's University, Kingston, Ont. K7L 3N6, Canada
SOURCE: Genomics, (1997) 45/2 (368-378).
Refs: 51
ISSN: 0888-7543 CODEN: GNMCEP
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Overexpression of multidrug-resistance protein (MRP) and P-glycoprotein confers similar but not identical multidrug-resistance phenotypes. However, unlike P-glycoprotein, which comprises two membrane-spanning domains (MSDs) and two nucleotide-binding domains, MRP contains a third NH2-proximal MSD, a feature now identified in several other ATP-binding cassette transmembrane transporters. MRP is located on chromosome 16 at band 13.1 close to the short-arm breakpoint of the pericentric inversion associated with the M4Eo subclass of acute myeloid leukemia. We have defined the intron-exon structure of MRP and characterized a number of splicing variants of MRP mRNA. The gene spans at least 200 kb. It contains 31 exons and a high proportion of class 0 introns, **alternative splicing** of which results in significant levels of variant transcripts that maintain the original open reading frame of MRP mRNA. Analyses of the conservation of intron-exon organization and protein

primary structure suggest that the MRP-related transporters evolved from a common ancestor shared with the **cystic fibrosis** transmembrane conductance regulator, by fusion with one or more genes encoding polytopic membrane proteins.

L12 ANSWER 13 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 11
ACCESSION NUMBER: 97148878 EMBASE
DOCUMENT NUMBER: 1997148878
TITLE: Sex lethal controls dosage compensation in Drosophila by a non-splicing mechanism.
AUTHOR: Kelley R.L.; Wang J.; Bell L.; Kuroda M.I.
CORPORATE SOURCE: M.I. Kuroda, Department of Cell Biology, Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, United States. mkuroda@bcm.tmc.edu
SOURCE: Nature, (1997) 387/6629 (195-199).
Refs: 25
ISSN: 0028-0836 CODEN: NATUAS
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Dosage compensation in Drosophila requires the male-specific lethal (msl) proteins (MSL) to make **gene** expression from the single male X chromosome equivalent to that from both female X chromosomes. Expression of msl2 is repressed post-transcriptionally by Sex lethal (SXL), a female-specific RNA-binding protein that regulates alternative splicing in the sex- determination hierarchy. Although msl2 RNA is alternatively spliced in males and females, this does not alter its coding potential and splicing is not required for male-specific expression of MSL2 protein. Instead, our results suggest that the association of SXL protein with multiple sites in the 5' and 3' untranslated regions of the mxl2 transcript represses its translation in females. Thus, this well characterized **alternative splicing factor** regulates at least one target transcript by a distinct mechanism.

L12 ANSWER 14 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 12
ACCESSION NUMBER: 97031790 EMBASE
DOCUMENT NUMBER: 1997031790
TITLE: The **alternative splicing factor** PSI regulates P-element third intron splicing in vivo.
AUTHOR: Adams M.D.; Tarng R.S.; Rio D.C.
CORPORATE SOURCE: D.C. Rio, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3204, United States
SOURCE: Genes and Development, (1997) 11/1 (129-138).
Refs: 34
ISSN: 0890-9369 CODEN: GEDEEP
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 021 Developmental Biology and Teratology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Splicing of the Drosophila P-element third intron (IVS3) is inhibited in somatic cells, restricting transposase expression to the germ line. Somatic inhibition of IVS3 splicing involves the assembly of a multiprotein complex on a regulatory sequence in the IVS3 5' exon. The P-element somatic inhibitor protein (PSI) is a component of this ribonucleoprotein complex and is required for inhibition of IVS3 splicing in vitro. The soma-specific expression pattern of PSI suggests that its low abundance in the germ line allows IVS3 splicing. We demonstrate that ectopic expression of PSI in the female germ line is sufficient to repress

splicing of an IVS3 reporter transgene. We also show that IVS3 splicing is activated in somatic embryonic cells in the presence of an antisense PSI ribozyme. These results support the model that PSI is a tissue-specific regulator of IVS3 splicing in vivo.

L12 ANSWER 15 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 13
ACCESSION NUMBER: 97332063 EMBASE
DOCUMENT NUMBER: 1997332063
TITLE: Identification of differentially expressed genes in chemically induced skin tumors.
AUTHOR: Rutberg S.E.; Lee E.J.; Hansen L.H.; Glick A.B.; Yuspa S.H.
CORPORATE SOURCE: S.H. Yuspa, Lab. of Cell. Carcin./Tumor Promo., National Cancer Institute, Bldg. 37, Bethesda, MD 20892, United States
SOURCE: Molecular Carcinogenesis, (1997) 20/1 (88-98).
Refs: 61
ISSN: 0899-1987 CODEN: MOCAE8
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
013 Dermatology and Venereology
016 Cancer
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Previous studies have demonstrated a role for the fos **gene** in promoting malignant conversion of mouse skin tumors. In the study reported here, differential display was performed to identify fos- and jun-regulated genes that are differentially expressed during premalignant progression. Total RNA isolated from variants of the papilloma cell line SP-1 transduced with retroviral vectors expressing v-jun and v-fos alone or in tandem was analyzed for the presence of differentially expressed transcripts by using 35 different primer combinations. Differentially expressed clones were rescreened by dot-blot analysis by using cDNA from chemically induced tumors with a high or low risk of malignant conversion.
Three differentially displayed fragments were isolated in this analysis. Homology searches indicated that these fragments shared significant homology with the apoptosis inhibitor bcl-2, human **alternative splicing factor/splicing factor** 2 (ASF/SF2), and a novel **gene** not present in the GenBank or EMBL databases. In situ hybridization indicated that the expression levels of the bcl-2 homolog increased with malignant potential in chemically derived mouse skin tumors. A similar analysis indicated that expression of the ASF/SF2 homolog was greater in papillomas than in normal skin or in squamous cell carcinomas. Transcripts for this **gene** were most abundant in the granular layer. The expression pattern of the third differential display fragment was consistent with that of a tumor suppressor **gene**. This **gene** was expressed at very high levels in normal skin and benign papillomas but was essentially undetectable in squamous cell carcinomas. Through this approach, we identified known and novel genes that may contribute to malignant progression in epidermal tumors.

L12 ANSWER 16 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 14
ACCESSION NUMBER: 1996:430202 CAPLUS
DOCUMENT NUMBER: 125:138569
TITLE: Both the wild type and a functional isoform of CFTR are expressed in kidney
AUTHOR(S): Morales, Marcelo M.; Carroll, Tiziana Piazza; Morita, Takashi; Schwiebert, Erik M.; Devuyst, Olivier;

Wilson, Patricia D.; Lopes, Anibal G.; Stanton, Bruce A.; Dietz, Harry C.; et al.
CORPORATE SOURCE: Dep. Physiol., Pediatrics, Med., Johns Hopkins Univ. Sch. Med., Baltimore, MD, 21205, USA
SOURCE: Am. J. Physiol. (1996), 270(6, Pt. 2), Fl038-F1048
CODEN: AJPHAP; ISSN: 0002-9513
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cystic fibrosis transmembrane conductance regulator (CFTR) consists of five domains, two transmembrane-spanning domains, each composed of six transmembrane segments, a regulatory domain, and two nucleotide-binding domains (NBDs). CFTR is expressed in kidney, but its role in overall renal function is not well understood, because mutations in CFTR found in patients with cystic fibrosis are not assocd. with renal dysfunction. To learn more about the distribution and functional forms of CFTR in kidney, we used a combination of mol., cell biol., and electrophysiol. approaches. These include an evaluation of CFTR mRNA and protein expression, as well as both two-electrode and patch clamping of CFTR expressed either in Xenopus oocytes or mammalian cells. In addn. to wild-type CFTR mRNA, an alternate form contg. only the first transmembrane domain (TMD), the first NBD, and the regulatory domain (TNR-CFTR) is expressed in kidney. Although missing the second set of TMDs and the second NBD, when expressed in Xenopus oocytes, TNR-CFTR has cAMP-dependent protein kinase A (PKA)-stimulated single Cl- channel characteristics and regulation of PKA activation of outwardly rectifying Cl- channels that are very similar to those of wild-type CFTR. TNR-CFTR mRNA is produced by an unusual mRNA processing mechanism and is expressed in a tissue-specific manner primarily in renal medulla.

L12 ANSWER 17 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 15
ACCESSION NUMBER: 1996:255989 CAPLUS
DOCUMENT NUMBER: 124:310430
TITLE: In vitro interaction between human immunodeficiency virus type 1 Rev protein and splicing factor ASF/SF2-associated protein, p32
AUTHOR(S): Tange, Thomas Oestergaard; Jensen, Torben Heick; Kjems, Joergen
CORPORATE SOURCE: Dep. Mol. Biol., Univ. Aarhus, Aarhus, DK-8000, Den.
SOURCE: J. Biol. Chem. (1996), 271(17), 10066-72
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Continuous replication of human immunodeficiency virus type 1 requires the expression of the regulatory protein Rev, which binds to the Rev response element (RRE) and up-regulates the cytoplasmic appearance of singly spliced and unspliced mRNA species. It has been demonstrated that the murine protein YL2 interacts with Rev in vivo and modulates the activity of Rev (Luo, Y., Yu, H., and Peterlin, B. M. (1994) J. Virol. 68, 3850-3856). Here we show that the YL2 human homolog, the p32 protein, which co-purifies with **alternative splicing factor** ASF/SF2, interacts directly with the basic domain of Rev in vitro and that the Rev-p32 complex is resistant to high concns. of salt or nonionic detergent. Protein footprinting data suggest that Rev interacts specifically with amino acids within the 196-208 region of p32. An anal. of the ternary complex, formed among p32, Rev, and RRE RNA, shows that Rev can bridge the assocn. of p32 and RRE. Furthermore, we demonstrate that exogenously added p32 specifically relieves the inhibition of splicing in vitro exerted by the basic domain of Rev. Our data are consistent with a

model in which p32 functions as a link between Rev and the cellular splicing app.

L12 ANSWER 18 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 16
ACCESSION NUMBER: 1996:393065 CAPLUS
DOCUMENT NUMBER: 125:108104
TITLE: Cystic fibrosis gene encodes a cAMP-dependent chloride channel in heart
AUTHOR(S): Hart, Padraig; Warth, John D.; Levesque, Paul C.; Collier, Mei Lin; Geary, Yvonne; Horowitz, Burton; Hume, Joseph R.
CORPORATE SOURCE: Department Physiology Cell Biology, University Nevada School Medicine, Reno, NV, 89557-0046, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1996), 93(13), 6343-6348
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CAMP-dependent chloride channels in heart contribute to autonomic regulation of action potential duration and membrane potential and have been inferred to be due to cardiac expression of the epithelial cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. In this report, a cDNA from rabbit ventricle was isolated and sequenced, which encodes an exon 5 splice variant (exon 5-) of CFTR, with >90% identity to human CFTR cDNA present in epithelial cells. Expression of this cDNA in Xenopus oocytes gave rise to robust cAMP-activated chloride currents that were absent in control water-injected oocytes. Antisense oligodeoxynucleotides directed against CFTR significantly reduced the d. of cAMP-dependent chloride currents in acutely cultured myocytes, thereby establishing a direct functional link between cardiac expression of CFTR protein and an endogenous chloride channel in native cardiac myocytes.

L12 ANSWER 19 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V. DUPLICATE 17
ACCESSION NUMBER: 96326203 EMBASE
DOCUMENT NUMBER: 1996326203
TITLE: Targeted disruption of an essential vertebrate **gene** : ASF/SF2 is required for cell viability.
AUTHOR: Wang J.; Takagaki Y.; Manley J.L.
CORPORATE SOURCE: Department of Biological Sciences, Columbia University, New York, NY 10027, United States
SOURCE: Genes and Development, (1996) 10/20 (2588-2599).
ISSN: 0890-9369 CODEN: GEDEEP
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Alternative splicing factor/splicing factor** 2 (ASF/SF2) is the prototype of a family of nuclear proteins highly conserved throughout metazoa, the SR (serine/arginine) proteins. Based largely on in vitro studies, SR proteins have been suggested to play important roles in constitutive and alternative splicing of pre-mRNAs. Here we describe the development of a genetic system employing the chicken B-cell line DT40 to study the function of ASF/SF2 in vivo. The high level of homologous recombination and rapid growth rate of these cells allowed us to show first that ASF/SF2 is an essential **gene**, and then to perform targeted disruption of both ASF/SF2 alleles, by creating a cell line in which the only source of ASF/SF2 is a human cDNA controlled by a tetracycline (tet)-repressible promoter. We show that addition of tet to these cells results in rapid depletion of ASF/SF2, concomitant accumulation of incompletely processed pre-mRNA, and

subsequent cell death. The tet- induced lethality could be rescued by plasmids expressing wild-type ASF/SF2, but not several mutant derivatives, or other SR proteins. Heterozygous cell lines overexpressing human ASF/SF2 displayed significant reductions of endogenous ASF/SF2 mRNA, suggesting that ASF/SF2 mRNA levels are controlled by an autoregulatory loop. This system provides a novel method for genetic analysis of factors that function in basic processes in vertebrate cells.

L12 ANSWER 20 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 18
ACCESSION NUMBER: 96046576 EMBASE
DOCUMENT NUMBER: 1996046576
TITLE: An intron enhancer recognized by splicing factors activates polyadenylation.
AUTHOR: Lou H.; Gagel R.F.; Berget S.M.
CORPORATE SOURCE: Verna/Marrs McClean Biochem. Dept., Baylor College of Medicine, Houston, TX 77030, United States
SOURCE: Genes and Development, (1996) 10/2 (208-219).
ISSN: 0890-9369 CODEN: GEDEEP
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Alternative processing of the pre-messenger RNA encoding calcitonin/calcitonin **gene**-related peptide (CT/CGRP) involves alternative inclusion of a 3'-terminal exon (exon 4) embedded within a six exon primary transcript. Expression of CT/CGRP in transgenic mice indicates that inclusion of exon 4 occurs in a wide variety of tissues, suggesting that the factors responsible for exon 4 inclusion are widely distributed. Inclusion of exon 4 requires an enhancer sequence located within the intron downstream of the poly(A) site of exon 4. Here we show that the intron enhancer activated in vitro polyadenylation cleavage of precursor RNAs containing the CT/CGRP exon 4 poly(A) site or heterologous poly(A) sites. To our knowledge this is the first example of an intron-located enhancer that facilitates polyadenylation. Within the enhancer sequence is a 5' splice site sequence immediately preceded by a pyrimidine tract. This 5' splice site sequence was required for enhanced polyadenylation and was recognized by both U1 small nuclear ribonucleoproteins (snRNPs) and **alternative splicing factor/splicing factor** 2 (ASF/SF2). Enhancement of polyadenylation required U1 RNA, suggesting that the 5' splice site sequence within the enhancer mediates enhancement via interaction with factors normally associated with functional 5' splice sites. Mutation of the polypyrimidine track of the enhancer also inhibited in vitro polyadenylation cleavage. Oligonucleotide competitions and UV cross-linking indicated that the enhancer pyrimidine track binds the polypyrimidine tract binding protein (PTB), but not U2 snRNP auxiliary factor (U2AF), and that binding of PTB was required for maximal enhancer-mediated polyadenylation. These results suggest that the enhancer binds known splicing factors, and that binding of these factors activates polyadenylation cleavage. Furthermore, these results suggest that regulation of alternative processing of CT/CGRP could occur at the level of polyadenylation, rather than splicing.

L12 ANSWER 21 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 19
ACCESSION NUMBER: 96208866 EMBASE
DOCUMENT NUMBER: 1996208866
TITLE: Analysis of **alternative splicing**

patterns in the **cystic fibrosis** transmembrane conductance regulator gene using mRNA derived from lymphoblastoid cells of **cystic fibrosis** patients.

AUTHOR: Bienvenu T.; Beldjord C.; Chelly J.; Fonknechten N.; Hubert D.; Dusser D.; Kaplan J.C.

CORPORATE SOURCE: Laboratoire de Biochimie Genetique, CHU Cochin, Pavillon Cassini, 123 boulevard de Port-Royal,F-75014 Paris, France

SOURCE: European Journal of Human Genetics, (1996) 4/3 (127-134). ISSN: 1018-4813 CODEN: EJHGEU

COUNTRY: Switzerland

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
022 Human Genetics

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Using in vitro amplification of cDNA by the polymerase chain reaction, we analyzed alternatively spliced events of **cystic fibrosis** transmembrane conductance regulator gene in lymphoblastoid cells. Ten alternatively spliced transcripts were identified using analysis of 6 overlapping segments of amplified cDNA, 4 of which have not been described previously. These include transcripts lacking exon 16, 17b, 22 and a transcript resulting from the use of a cryptic acceptor and donor splice sites. Moreover, in 2 **cystic fibrosis** (CF) patients bearing nonsense mutations E60X or W1282X, we observed that nonsense mutations are associated with an alteration of splice site selection in vivo resulting in exon skipping of constitutive exons or in the use of cryptic splice sites. In addition, even though lymphoblastoid cells are not the relevant tissue to address the question of the relationship between clinical respiratory phenotype and genotype, our results concerning adult CF patients (.DELTA.F508/.DELTA.F508) suggest that individual-specific RNA splicing patterns could influence the severity of the CF pulmonary disease. If this phenomenon of **alternative splicing** events proves to be significant in CF and to be a common feature of disease genes, the study of RNA splicing could become an important tool for the analysis of the genotype-phenotype relationship in many inherited disorders.

L12 ANSWER 22 OF 45 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:820224 CAPLUS

DOCUMENT NUMBER: 123:333108

TITLE: Identification and characterization of three members of the human SR family of pre-mRNA splicing factors

AUTHOR(S): Screaton, Gavin R.; Caceres, Javier F.; Mayeda, Akila; Bell, Martyn V.; Plebanski, Magda; Jackson, David G.; Bell, John I.; Krainer, Adrian R.

CORPORATE SOURCE: Mol. Immunol. Group, John Radcliffe Hops., Oxford, OX3 9DU, UK

SOURCE: EMBO J. (1995), 14(17), 4336-49 CODEN: EMJODG; ISSN: 0261-4189

DOCUMENT TYPE: Journal

LANGUAGE: English

AB SR proteins have a characteristic C-terminal Ser/Arg-rich repeat (RS domain) of variable length and constitute a family of highly conserved nuclear phosphoproteins that can function as both essential and alternative pre-mRNA splicing factors. The authors have cloned a cDNA encoding a novel human SR protein designated SRp30c, which has an unusually short RS domain. The authors also cloned cDNAs encoding the human homologues of Drosophila SRp55/B52 and rat SRp40/HRS. Recombinant proteins expressed from these cDNAs are active in constitutive splicing,

as shown by their ability to complement a HeLa cell S100 ext. deficient in SR proteins. Addnl. cDNA clones reflect extensive alternative splicing of SRp40 and SRp55 pre-mRNAs. The predicted protein isoforms lack the C-terminal RS domain and might be involved in feedback regulatory loops. The ability of human SRp30c, SRp40 and SRp55 to modulate alternative splicing in vivo was compared with that of other SR proteins using a transient cotransfection assay. The overexpression of individual SR proteins in HeLa cells affected the choice of alternative 5' splice sites of adenovirus E1A and/or human .beta.-thalassemia reporters. The resulting splicing patterns were characteristic for each SR protein. Consistent with the postulated importance of SR proteins in alternative splicing in vivo, the authors demonstrate complex changes in the levels of mRNAs encoding the above SR proteins upon T cell activation, concomitant with changes in the expression of alternatively spliced isoforms of CD44 and CD45.

L12 ANSWER 23 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94346242 EMBASE
DOCUMENT NUMBER: 1994346242
TITLE: Purification and characterization of a kinase specific for the serine- and arginine-rich pre-mRNA splicing factors.
AUTHOR: Gui J.-F.; Tronchere H.; Chandler S.D.; Fu X.-D.
CORPORATE SOURCE: Cellular/Molecular Medicine Division, University of California, 9500 Gilman Drive,San Diego, CA 92093-0651, United States
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994) 91/23 (10824-10828). ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Members of the SR family of pre-mRNA splicing factors are phosphoproteins that share a phosphoepitope specifically recognized by monoclonal antibody (mAb) 104. Recent studies have indicated that phosphorylation may regulate the activity and the intracellular localization of these splicing factors. Here, we report the purification and kinetic properties of SR protein kinase 1 (SRPK1), a kinase specific for SR family members. We demonstrate that the kinase specifically recognizes the SR domain, which contains serine/arginine repeats. Previous studies have shown that dephosphorylated SR proteins did not react with mAb 104 and migrated faster in SDS gels than SR proteins from mammalian cells. We show that SRPK1 restores both mobility and mAB 104 reactivity to a SR protein SF2/ASF (splicing factor 2/**alternative splicing factor**) produced in bacteria, suggesting that SRPK1 is responsible for the generation of the mAb 104-specific phosphoepitope in vivo. Finally, we have correlated the effects of mutagenesis in the SR domain of SF2/ASF on splicing with those on phosphorylation of the protein by SRPK1, suggesting that phosphorylation of SR proteins is required for splicing.

L12 ANSWER 24 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 20
ACCESSION NUMBER: 94158432 EMBASE
DOCUMENT NUMBER: 1994158432
TITLE: Cellular protein modulates effects of human immunodeficiency virus type 1 Rev.
AUTHOR: Luo Y.; Yu H.; Peterlin B.M.
CORPORATE SOURCE: Department of Medicine, Howard Hughes Medical Institute,

University of California, 3rd and Parnassus Ave.,San Francisco, CA 94143-0724, United States
SOURCE: Journal of Virology, (1994) 68/6 (3850-3856).
ISSN: 0022-538X CODEN: JOVIAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Replication of human immunodeficiency virus type 1 requires expression of the viral trans activator Rev. Rev binds to a highly structured RNA, the Rev response element, which is present in singly spliced and unspliced genomic viral RNAs. Although Rev helps to transport these transcripts from the nucleus to the cytoplasm, the mechanism(s) involved is not fully understood. Using the yeast two-hybrid system, we isolated a murine protein (YL2) that interacts with the basic domain of Rev, which is essential for the function of Rev in vivo and for the inhibitory splicing activity of Rev in vitro. YL2 has 92% identity to a human 32-kDa protein (p32), which copurifies with **alternative splicing factor** SF2/ASF. Furthermore, we found that whereas expression of YL2 greatly potentiated the activity of Rev, antisense YL2 transcripts blocked the effects of Rev in mammalian cells. YL2 also increased the activities of Rex on the Rex response element and of hybrid Rev proteins fused to Tat and the coat protein of bacteriophage MS2 on their respective RNAs. Thus, YL2 or p32 is a cellular protein that modulates the function of human immunodeficiency virus type 1 Rev.

L12 ANSWER 25 OF 45 MEDLINE
ACCESSION NUMBER: 95047327 MEDLINE
DOCUMENT NUMBER: 95047327
TITLE: Regulation of tissue-specific P-element pre-mRNA splicing requires the RNA-binding protein PSI.
AUTHOR: Siebel C W; Kanaar R; Rio D C
CORPORATE SOURCE: Department of Molecular and Cell Biology, University of California, Berkeley 94720.
CONTRACT NUMBER: 5R01-HD28063-04 (NICHD)
SOURCE: GENES AND DEVELOPMENT, (1994 Jul 15) 8 (14) 1713-25.
Journal code: FN3. ISSN: 0890-9369.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502

AB Binding of a multiprotein complex to a 5' exon inhibitory element appears to repress splicing of the Drosophila P-element third intron (IVS3) in soma. We have purified 97- and 50-kD proteins that interact specifically with the inhibitory element using RNA affinity chromatography. Antibodies specific for the 97-kD protein relieve inhibition of IVS3 splicing in somatic extracts, providing direct evidence that inhibition requires this protein, P-element somatic inhibitor (PSI). We identify the 50-kD protein as hrp48, a protein similar to the mammalian splicing factor hnRNP A1, and show that hrp48 recognizes specific nucleotides in a pseudo-5' splice site within the inhibitory element. The results indicate that PSI is an **alternative splicing factor** that regulates tissue-specific splicing, probably through interactions with generally expressed factors such as hrp48.

L12 ANSWER 26 OF 45 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1994:623590 CAPLUS
DOCUMENT NUMBER: 121:223590

TITLE: Regulation of alternative splicing in vivo by overexpression of antagonistic splicing factors
AUTHOR(S): Caceres, Javier F.; Stamm, Stefan; Helfman, David M.; Krainer, Adrian R.
CORPORATE SOURCE: Colod Spring Harbor Lab., Cold Spring Harbor, NY, 11724, USA
SOURCE: Science (Washington, D. C.) (1994), 265(5179), 1706-9
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The opposing effects of SF2/ASF and heterogeneous nuclear ribonucleoprotein (hnRNP) A1 influence alternative splicing in vitro. SF2/ASF or hnRNP A1 complementary DNAs were transiently overexpressed in HeLa cells, and the effect on alternative splicing of several co-transfected reporter genes was measured. Increased expression of SF2/ASF activated proximal 5' splice sites, promoted inclusion of a neuron-specific exon, and prevented abnormal exon skipping. Increased expression of hnRNP A1 activated distal 5' splice sites. Therefore, variations in the intracellular levels of antagonistic splicing factors influence different modes of alternative splicing in vivo and may be a natural mechanism for tissue-specific or developmental regulation of **gene** expression.

L12 ANSWER 27 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 21
ACCESSION NUMBER: 1994:526626 CAPLUS
DOCUMENT NUMBER: 121:126626
TITLE: Analysis of mutations and alternative splicing patterns in the CFTR gene using mRNA derived from nasal epithelial cells
AUTHOR(S): Hull, Jeremy; Shackleton, Sue; Harris, Ann
CORPORATE SOURCE: Institute of Molecular Medicine, Oxford University, Oxford, OX3 9DU, UK
SOURCE: Hum. Mol. Genet. (1994), 3(7), 1141-6
CODEN: HMGEE5; ISSN: 0964-6906
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Ten to fifteen percent of cystic fibrosis (CF) chromosomes carry mutations which are not detected by routine screening of the CFTR gene for known mutations. Many techniques have been used to screen the CFTR gene for these remaining mutations. Most of the methods use genomic DNA, and since the CFTR gene contains 27 exons, are necessarily labor intensive. The authors have screened the entire coding region of CFR, by chem. cleavage of 7 overlapping segments of amplified cDNA. Using this method the authors have identified 4 sequence changes which had not been detected by screening genomic DNA, and successfully detected 10 out of 13 known mutations. In addn., the authors have identified 8 alternatively spliced forms of CFTR mRNA, 4 of which have not been described previously. These include transcripts lacking (a) exon 3, (b) exons 2 + 3, (c) exons 9 + 12, and (d) the final 357 bp of exon 15 as a result of use of the cryptic splice donor site CA2863/GTTCGT.

L12 ANSWER 28 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 22
ACCESSION NUMBER: 1994:673630 CAPLUS
DOCUMENT NUMBER: 121:273630
TITLE: Alternative splicing of the first nucleotide binding fold of CFTR in mouse testes is associated with specific stages of spermatogenesis
AUTHOR(S): Delaney, Stephen J.; Koopman, Peter; Lovelock, Paul K.; Wainwright, Brandon J.
CORPORATE SOURCE: Cent. Mol. Biol. Biotechnol., Univ. Queensland, Brisbane, 4072, Australia
SOURCE: Genomics (1994), 20(3), 517-18

CODEN: GNMCEP; ISSN: 0888-7543
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The alternatively spliced CFTR exon 11b+ transcripts were produced predominantly, if not exclusively, in the germ cells of the murine testis.
Alternative splicing of exon 11b+ coincided with the germ cell stage at which the majority of CFTR transcription occurred.

L12 ANSWER 29 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 23
ACCESSION NUMBER: 94025994 EMBASE
DOCUMENT NUMBER: 1994025994
TITLE: Exon 9 of the CFTR gene: Splice site haplotypes and cystic fibrosis mutations.
AUTHOR: Dork T.; Fislage R.; Neumann T.; Wulf B.; Tummler B.
CORPORATE SOURCE: Abteilung Biophysikalische Chemie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Strasse 8,D-30625 Hannover, Germany
SOURCE: Human Genetics, (1994) 93/1 (67-73).
ISSN: 0340-6717 CODEN: HUGEDQ
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
021 Developmental Biology and Teratology
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The alternatively spliced exon 9 of the **cystic fibrosis** transmembrane conductance regulator (CFTR) gene codes for the initial part of the amino-terminal nucleotide-binding fold of CFTR. A unique feature of the acceptor splice site preceding this exon is a variable length polymorphism within the polypyrimidine tract influencing the extent of exon 9 skipping in CFTR mRNA. We investigated this repeat for its relationship to CFTR mutations and intragenic markers on 200 chromosomes from German patients with **cystic fibrosis** (CF). Four frequent length variations were strongly associated with the four predominant haplotypes previously defined by intragenic marker dimorphisms. One of these alleles displayed absolute linkage disequilibrium to the major CF mutation .DELTA.F508. Other frequent CFTR mutations were linked to one particular splice site haplotype indicating that differential exon 9 skipping contributes little to the clinical heterogeneity among CF patients with an identical mutation. We also identified a novel missense mutation (V456F) and a novel nonsense mutation (Q414X) within the coding region of exon 9. The missense mutation V456F adjacent to walker motif A was present in a pancreas-sufficient CF patient. In contrast, the pancreas-insufficient Q414X/.DELTA.F508 compound heterozygote suffered from a severe form of the disease, indicating that **alternative splicing** of exon 9 does not overcome the deleterious effect of a stop codon within this exon.

L12 ANSWER 30 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 24
ACCESSION NUMBER: 1993:556834 CAPLUS
DOCUMENT NUMBER: 119:156834
TITLE: Alternative splicing of CFTR chloride channels in heart
AUTHOR(S): Horowitz, Burton; Tsung, Stephen S.; Hart, Padraig; Levesque, Paul C.; Hume, Joseph R.
CORPORATE SOURCE: Sch. Med., Univ. Nevada, Reno, NV, 89557, USA
SOURCE: Am. J. Physiol. (1993), 264(6, Pt. 2), H2214-H2220
CODEN: AJPHAP; ISSN: 0002-9513
DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors amplified cDNA from rabbit ventricle and cloned fragments corresponding to the 12 transmembrane spanning domains of the epithelial CFTR transcript. The deduced sequence from rabbit heart indicated deletion of a 30-amino acid segment in the 1st cytoplasmic loop of CFTR, which corresponds to known locations of intron-exon junctions bordering exon 5 in the CFTR gene, suggesting that CFTR is alternatively spliced in heart. Outside this region, the heart CFTR isoform displayed >95% identity to human epithelial CFTR. Mol. anal. demonstrated CFTR expression only in cardiac tissues that exhibited a cAMP-dependent Cl- conductance in native cells. The expression of a specific isoform of CFTR Cl- channels in mammalian heart may have functional and clin. significance.

L12 ANSWER 31 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 25
ACCESSION NUMBER: 1993:536176 CAPLUS
DOCUMENT NUMBER: 119:136176
TITLE: Testis-specific, alternative splicing of rodent CFTR mRNA
AUTHOR(S): Trezise, Ann E. O.; Buchwald, Manuel; Higgins, Christopher F.
CORPORATE SOURCE: John Radcliffe Hosp., Univ. Oxford, Oxford, OX3 9DU, UK
SOURCE: Hum. Mol. Genet. (1993), 2(6), 801-2
CODEN: HMGEE5; ISSN: 0964-6906
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In this communication the authors describe the testis-specific, alternative splicing of CFTR mRNA in rodents. By Northern blot anal. a single transcript, approx. 6.0 kb in length, was identified in parotid gland, submaxillary gland, uterus, pancreas, trachea, lung, stomach, duodenum, jejunum, proximal colon and distal colon (data not shown in rat and/or mouse. In contrast, Northern blot anal. of mouse and rat testis RNA reveals 2 mRNA transcripts corresponding to CFTR coding sequences. One transcript was approx. 6 kb in length, similar to the transcript seen in all other cells and tissues examd. The 2nd, testis-specific transcript, approx. 1 kb larger, was found in RNA isolated from the testes of both mouse and rat. In the mouse testes, both transcripts were present in approx. equal amts., while in rat testes the larger transcript predominated.

L12 ANSWER 32 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 26
ACCESSION NUMBER: 1993:557607 CAPLUS
DOCUMENT NUMBER: 119:157607
TITLE: [illegible] mutations in the CFTR gene
AUTHOR(S): Hull, Jeremy; Shackleton, Sue; Harris, Ann
CORPORATE SOURCE: Inst. Mol. Med., John Radcliffe Hosp., Oxford, OX3 9DU, UK
SOURCE: Hum. Mol. Genet. (1993), 2(6), 689-92
CODEN: HMGEE5; ISSN: 0964-6906
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Three different putative splicing mutations in the CFTR gene have been studied by analyzing mRNA extd. from nasal epithelial cells harvested from patients with cystic fibrosis. Six patients were analyzed, all of whom had classical symptoms of cystic fibrosis (CF). Two patients carried the 621 + 1G .fwdarw. T mutation, 3 patients carried the 1717 - 1G .fwdarw. A mutation and 1 patient carried the 1898 + 1G .fwdarw. A mutation. All patients carried the .DELTA.F508 mutation on the other chromosome. Ten non-CF control subjects were also studied. The 621 + 1G .fwdarw. T

mutation resulted in activation of an alternative splice site within exon 4 in one patient and activation of this site or skipping of exon 4 in the other patient. The 1717 - 1G .fwdarw. A mutation resulted in skipping of exon 11 in all 3 patients studied and the 1898 + 1G .fwdarw. T mutation resulted in skipping of exon 12. These expts. demonstrate that these mutations do result in aberrant splicing of CFTR mRNA as predicted from the changes in genomic sequence.

L12 ANSWER 33 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 27
ACCESSION NUMBER: 93021377 EMBASE
DOCUMENT NUMBER: 1993021377
TITLE: **Alternative splicing** of intron 23 of the human **cystic fibrosis** transmembrane conductance regulator gene resulting in a novel exon and transcript coding for a shortened intracytoplasmic C terminus.
AUTHOR: Yoshimura K.; Chin Shyan Chu; Crystal R.G.
CORPORATE SOURCE: Pulmonary Branch, NHLBI, National Institutes of Health, Bethesda, MD 20892, United States
SOURCE: Journal of Biological Chemistry, (1993) 268/1 (686-690). ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The **cystic fibrosis** transmembrane conductance regulator (CFTR) gene, the gene responsible for the lethal hereditary disorder **cystic fibrosis**, codes for a membrane protein functioning as a cAMP-regulated Cl- channel. Evaluation of human CFTR mRNA transcripts from epithelial and nonepithelial cells demonstrated a CFTR cDNA containing a 260-base pair (bp) insertion between the known CFTR exons 23 and 24, introducing a premature stop codon that would result in a CFTR protein shortened by 61 amino acids at the carboxyl terminus compared to that expected from the normal reported human CFTR coding sequences. Sequence analysis of intron 23 of the CFTR gene demonstrated that the 260-bp insertion (named exon 24a), a part of the reported intron 23 and located consecutive to exon 24, is likely generated by an alternative splice acceptor site. The exon 24a+ CFTR mRNA transcripts represented 3-16% of the total CFTR transcripts in epithelial and nonepithelial cells. These observations suggest an unexpected plasticity of expression of the CFTR gene, where **alternative splicing** of precursor [illegible] a genomic segment previously believed to function as an intron.

L12 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 28
ACCESSION NUMBER: 1993:557606 CAPLUS
DOCUMENT NUMBER: 119:157606
TITLE: Analysis of CFTR transcripts in nasal epithelial cells and lymphoblasts of a cystic fibrosis patient with 621 + 1G .fwdarw. T and 711 + 1G .fwdarw. T mutations
AUTHOR(S): Zielenski, Julian; Bozon, Dominique; Markiewicz, Danuta; Aubin, Gervais; Simard, Fernand; Rommens, Johanna M.; Tsui, Lap Chee
CORPORATE SOURCE: Dep. Genet., Hosp. Sick Child., Toronto, ON, M5G 1X8, Can.
SOURCE: Hum. Mol. Genet. (1993), 2(6), 683-7 CODEN: HMGEE5; ISSN: 0964-6906

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have analyzed the CFTR mRNA populations in a cystic fibrosis patient heterozygous for the 621 + 1G .fwdarw. T and 711 + 1G .fwdarw. T mutations. Total RNA isolated from the nasal epithelial cells and Epstein-Barr virus-transformed lymphoblasts derived from this patient was reversely transcribed and a region extending from exon 3 to exon 7 of the gene was amplified by the polymerase chain reaction and analyzed. Three abnormal products were identified, suggesting the presence of three aberrant transcripts, and their profiles were identical in both cell types. Two of the products were found to be missing either exon 4 or exon 5 as anticipated from the transcripts from the 621 + 1G .fwdarw. T or 711 + 1G .fwdarw. T alleles, resp. The third product was apparently derived from an alternatively spliced mRNA species in the absence of the nominal splice site (in 621 + 1G .fwdarw. T) through the use of a cryptic splice donor sequence (TT528/GTGAGG) within exon 4. Although reading frames appeared to be preserved in all three putative transcripts, significant portions of the presumed first and second transmembrane spans as well as the immediately following cytoplasmic domain would be deleted from the mutant CFTR polypeptides, if made. These observations are consistent with a loss of CFTR function in this cystic fibrosis patient.

L12 ANSWER 35 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 29
ACCESSION NUMBER: 1993:209935 CAPLUS
DOCUMENT NUMBER: 118:209935
TITLE: Alternative splicing in the first nucleotide binding fold of CFTR
AUTHOR(S): Will, Katrin; Stuhrmann, Manfred; Dean, Michael; Schmidtke, Joerg
CORPORATE SOURCE: Inst. Hum. Genet., Med. Hochsch. Hannover, Hannover, 3000/61, Germany
SOURCE: Hum. Mol. Genet. (1993), 2(3), 231-5
CODEN: HMGEE5; ISSN: 0964-6906
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CFTR mRNA transcripts were analyzed from freshly isolated nasal epithelial cells and lymphocytes (six individuals) and from lymphocytes alone from 14 further individuals. In four of these 20 individuals alternative splicing was obsd. within the region coding for the first nucleotide binding fold. The RNA sequence between exons 10 and 13 was converted to cDNA and amplified by the polymerase chain reaction (PCR). Two PCR products of 583 bp and 101 bp were detected. Direct sequencing of both fragments showed that the 583 bp PCR fragment contained an addnl. 119 bp sequence between exon 10 and exon 11, directly at the normal junction. This insertion contains an in frame stop codon and would, if translated, cause a shift in the reading frame. This stop codon does not result in an undetectable mRNA level as seen with other nonsense mutations within the same region of the CFTR gene. The alternatively spliced mRNA was found to be transcribed from both CF and normal alleles. The 119 bp fragment was amplified from genomic DNA and from the genomic phage TE24V, which includes exon 9, intron 9, exon 10 and a part of intron 10 by PCR using primers created from within the inserted sequence. In addn., the insertion was mapped to a 1 Kb EcoRI fragment of phage TE24V by Southern-blot anal. Sequencing the insert surroundings within the phage TE24V revealed consensus splice sites (donor and acceptor sites, branch point). Furthermore no alterations were detected in the splice site sequences between individuals

who express the aberrantly spliced product and those who do not.

L12 ANSWER 36 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 30
ACCESSION NUMBER: 93248284 EMBASE
DOCUMENT NUMBER: 1993248284
TITLE: Alternative splicing of a previously unidentified CFTR exon
introduces an in-frame stop codon 5' of the R region.
AUTHOR: Melo C.A.; Serra C.; Stoyanova V.; Aguzzoli C.; Faraguna D.; Tamanini A.; Berton G.; Cabrini G.; Baralle F.E.
CORPORATE SOURCE: International Center for Genetic, Engineering and Biotechnology, Area Science Park, Padriciano 99,34012 Trieste, Italy
SOURCE: FEBS Letters, (1993) 329/1-2 (159-162).
ISSN: 0014-5793 CODEN: FEBLAL
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The **cystic fibrosis** transmembrane conductance regulator (CFTR) has been extensively characterized as the carrier of the basic defect in **cystic fibrosis**. CFTR is part of a growing family of proteins encoded by a single gene, the variant isoforms of which are generated by **alternative splicing** or RNA editing. We have analyzed the CFTR mRNA in the region of exons 10-11 in T84 cells and detected an alternatively spliced exon (10b) accounting for about 5% of the CFTR mRNA. The exon 10b found in both the human and mice genomes, introduces an in-frame stop codon. The resulting mRNA is translated into a truncated CFTR protein, identified in T84 cells by immunoprecipitation with the CFTR-specific monoclonal antibody MATG 1061. The insertion of a differentially spliced exon carrying an in-frame stop codon is a novel cellular mechanism for the production of a protein sharing common sequences with another, but having different properties and functions.

L12 ANSWER 37 OF 45 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1993:619734 CAPLUS
DOCUMENT NUMBER: 119:219734
TITLE: Specific commitment of different pre-mRNAs to splicing by single SR proteins
AUTHOR(S): Fu, Xiang Dong
CORPORATE SOURCE: Div. Cell. Mol. Med., Univ. California, San Diego, La Jolla, CA, 92093-0651, USA
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Higher eukaryotic cells express a family of essential splicing factors with a characteristic RNA-binding domain and serine/arginine-rich (SR) motif. These SR proteins, which include SC35 and SF2/ASF, are conserved from Drosophila to man, are required for early steps of spliceosome assembly, and can influence splice-site selections. To address their mechanisms of action, SR proteins are examd. for their role in committing pre-mRNA to the splicing pathway. SC35 was sufficient on its own to form a committed complex with human .beta.-globin pre-mRNA. Examn. of other SR proteins and pre-mRNA substrates revealed that single SR proteins committed different pre-mRNAs to splicing with pronounced substrate specificity. These results suggest that splicing of different pre-mRNAs may require distinct sets of SR proteins, and that the commitment by SR proteins may be a crit. step at which alternative and tissue-specific

splicing is regulated.

L12 ANSWER 38 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 31
ACCESSION NUMBER: 1992:528703 CAPLUS
DOCUMENT NUMBER: 117:128703
TITLE: Regulation of cystic fibrosis transmembrane conductance regulator (CFTR) gene transcription and alternative RNA splicing in a model of developing intestinal epithelium
AUTHOR(S): Montrose-Rafizadeh, Chahrzad; Blackmon, Denise L.; Hamosh, Ada; Oliva, Maria M.; Hawkins, Anita L.; Curristin, Sheila M.; Griffin, Constance A.; Yang, Vincent W.; Guggino, William B.; et al.
CORPORATE SOURCE: Sch. Med., Johns Hopkins Univ., Baltimore, MD, 21205, USA
SOURCE: J. Biol. Chem. (1992), 267(27), 19299-305
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Transcriptional and post-transcriptional regulation of CFTR (cystic fibrosis transmembrane conductance regulator) gene expression was studied in HT29 cells. It is known that the abundance of CFTR mRNA increases during differentiation of pluripotent HT29-18 cells and is maintained at high levels in the stably differentiated HT29-18C1 subclone. Nuclear run-on assays suggest that increased transcription of the CFTR gene explains the increased abundance of total CFTR mRNA in differentiated HT29 cells. The increased transcription cannot be ascribed to cell cycle-dependent expression of the CFTR gene or to changes in CFTR gene copy no. between subcloned cells. Similar to native tissue cells, differentiated HT29 cells contain low copy nos. of CFTR transcripts (1-5/cell), and a portion of the CFTR transcripts are alternatively spliced to remove exon 9 (and make 9- mRNA). During differentiation of HT29-18 cells, the abs. amt. of full-length CFTR mRNA increases 8-fold, whereas the amt. of 9- mRNA increases 18-fold. The fraction of 9- mRNA in the CFTR mRNA pool is increased in differentiated HT29 cells. The results show that gene transcription regulates the abundance of CFTR transcripts and that regulatory control of alternative RNA splicing may also be a cellular mechanism to modulate CFTR function.

L12 ANSWER 39 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V. DUPLICATE 32
ACCESSION NUMBER: 92126107 EMBASE
DOCUMENT NUMBER: 1992126107
TITLE: A potential splicing factor is encoded by the opposite strand of the trans-spliced c-myb exon.
[illegible] Bureau A.; Boiet C.; Martinerie C.; Perbal B.
CORPORATE SOURCE: Lab. d'Oncologie Virale Moleculaire, Centre Universitaire, Institut Curie, 91405 Orsay Cedex, France
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1992) 89/7 (2511-2515).
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We previously established that the expression of a thymic c-myb mRNA species requires the intermolecular recombination of coding sequences expressed from transcriptional units localized on different chromosomes, in both chicken and human. We now report that a putative splicing factor (PR264), extremely well conserved in chicken and human, is encoded by the opposite strand of the c-myb trans-spliced exon. The PR264 polypeptide, which contains a typical ribonucleoprotein 80 and an arginine/serine-rich

domain, is highly homologous to the Drosophila splicing regulators tra, tra-2, and su(wa) and to the human **alternative splicing factor** ASF/SF2. Furthermore, we show that PR264-specific mRNAs are expressed in normal hematopoietic cells of chicken and human origin and that the relative proportion of the PR264 transcripts is developmentally regulated in chicken.

L12 ANSWER 40 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 33
ACCESSION NUMBER: 92272387 EMBASE
DOCUMENT NUMBER: 1992272387
TITLE: Omission of exon 12 in cystic fibrosis transmembrane conductance regulator (CFTR) gene transcripts.
AUTHOR: Slomski R.; Schloesser M.; Berg L.-P.; Wagner M.; Kakkar V.V.; Cooper D.N.; Reiss J.
CORPORATE SOURCE: Ins fur Humangenetik der Universitat, Gosslerstrasse 12d,W-3400 Gottingen, Germany
SOURCE: Human Genetics, (1992) 89/6 (615-619).
ISSN: 0340-6717 CODEN: HUGEDQ
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Cystic fibrosis** transmembrane conductance regulator (CFTR) mRNA transcripts isolated from both expressing and 'non-expressing' cell types of normal individuals exhibit differntial splicing to a variable extent in a region encoding the putative nucleotide binding fold of the CFTR polypeptide. Sequence analysis of the aberrant fragments obtained after cDNA polymerase chain reaction amplification confirmed the in-frame joining of exons 11 and 13. The proportion of **alternative splicing** is reproducible and constant in a given individual. The omission of exon 12 in a significant proportion of transcripts supports the hypothesis that a minimal amount of correctly expressed CFTR is sufficient for the maintenance of a clinically normal phenotype.

L12 ANSWER 41 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 34
ACCESSION NUMBER: 92164318 EMBASE
DOCUMENT NUMBER: 1992164318
TITLE: Quantitative expression patterns of multidrug-resistance P-glycoprotein (MDR1) and differentially spliced cystic-fibrosis transmembrane-conductance regulatory mRNA transcripts in human epithelia.
AUTHOR: Bremer S.; Hoof T.; Wilke M.; Busche R.; Scholte B.; Riordan J.R.; Maass G.; Tummler B.
CORPORATE SOURCE: Abt. Biophysikalische Chemie, Medizinische Hochschule, Konstanty Gutschow Str. 8,W-3000 Hannover 61, Germany
SOURCE: European Journal of Biochemistry, (1992) 206/1 (137-149).
ISSN: 0014-2956 CODEN: EJBCAI
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB P-glycoprotein (MDR1), that confers multidrug resistance in cancer, and the **cystic-fibrosis** transmembrane-conductance regulator (CFTR), that is causative defective in **cystic fibrosis**, belong to the family of ATP-binding transport proteins. The expression of MDR1 and CFTR in human epithelial tissues and the cell lines T84 and HT29 was estimated by primer-directed reverse transcription (RT) and subsequent monitoring of the kinetics of cDNA product formation during the polymerase chain reaction (PCR). MDR1 mRNA was found in high levels, 15-50 amol mRNA/.mu.g RNA, in the intestine, kidney, liver and placenta, and in low levels, 0.2 amol/.mu.g RNA, in respiratory

epithelium. Large amounts of CFTR mRNA were measured in the gastrointestinal tract, whereas the kidney, as the phenotypically normal organ, and the lung, as the most severely affected organ in **cystic fibrosis**, both contained low amounts, 3 amol CFTR/.mu.g RNA. CFTR transcript levels of 1-5 amol/.mu.g RNA were determined in lymphocytes and lymphoblast cell lines, suggesting that lymphoblasts are an accessible source for the study of the molecular pathogenesis of **cystic fibrosis**. When transcripts were scanned by overlapping RT/PCR analyses, only transcript of expected size was detected for MDR1 mRNA, whereas variable in-frame deletions of either exon 4, 9 or 12 were observed in CFTR mRNA. The complete loss of single exons was seen at proportions of 1-40% in all investigated tissues and cell lines with large donor-to-donor variation. Exons 9 and 12 of the CFTR gene encode parts of the evolutionarily well-conserved first nucleotide-binding fold including the two Walker motifs. **Alternative splicing** may give rise to various CFTR forms of different function and localization.

L12 ANSWER 42 OF 45 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1993:664114 CAPLUS
DOCUMENT NUMBER: 119:264114
TITLE: Multiple activities of the human splicing factor ASF
AUTHOR(S): Harper, Joan E.; Manley, James L.
CORPORATE SOURCE: Dep. Biol. Sci., Columbia Univ., New York, NY, USA
SOURCE: Gene Expression (1992), 2(1), 19-29
CODEN: GEEXEJ; ISSN: 1052-2166
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effects of human **alternative splicing factor**, ASF, on in vitro splicing of adenovirus E1A pre-mRNA were examd. E1A pre-mRNA is a complex substrate, and splicing in HeLa cell nuclear exts. produces six different RNAs using three alternative 5' splice sites and two 3' splice sites. Addn. of excess ASF to splicing reactions produced a simplified splicing pattern, in which only one spliced product, 13S RNA, was detected. Inhibition of 12S and 9S splicing, which use 5' splice sites upstream of the 13S 5' splice site, extends previous observations that when multiple 5' splice sites compete for the same 3' splice site, ASF causes preferential selection of the proximal 5' splice site. However, inhibition of the other splices, which use a different upstream 3' splice site, represents a novel activity of ASF, as competition between 5' splice sites is not involved. The effect of ASF on 12S splicing was found to depend on its position relative to competing 5' splice sites, indicating that the ability of ASF to activate proximal 5' splice sites is position- but not sequence-dependent. Finally, addn. of small amts. of ASF to ASF-lacking S100 ext. was able to activate distal as well as proximal 5' splice sites in two of three pre-mRNAs tested, indicating that in these cases changes in the concn. of ASF alone can be sufficient to modulate alternative 5' splice site selection.

L12 ANSWER 43 OF 45 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1992:17285 CAPLUS
DOCUMENT NUMBER: 116:17285
TITLE: A novel protein factor is required for use of distal alternative 5' splice sites in vitro
AUTHOR(S): Harper, Joan E.; Manley, James L.
CORPORATE SOURCE: Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724, USA
SOURCE: Mol. Cell. Biol. (1991), 11(12), 5945-53
CODEN: MCEBD4; ISSN: 0270-7306
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Adenovirus E1A pre-mRNA was used as a model to examine alternative 5' splice site selection during in vitro splicing reactions. Strong

preference for the downstream 13S 5' splice site over the upstream 12S or 9S 5' splice sites was obsd. However, the 12S 5' splice site was used efficiently when a mutant pre-mRNA lacking the 13S 5' splice site was processed, and 12S splicing from this substrate was not reduced by 13S splicing from a sep. pre-mRNA, demonstrating that 13S splicing reduced 12S 5' splice site selection through a bona fide cis-competition. DEAE-cellulose chromatog. of nuclear ext. yielded 2 fractions with different splicing activities. The bound fraction contained all components required for efficient splicing of simple substrates but was unable to utilize alternative 5' splice sites. In contrast, the flow-through fraction, which by itself was inactive, contained an activity required for alternative splicing and was shown to stimulate 12S and 9S splicing, while reducing 13S splicing, when added to reactions carried out by the bound fraction. Furthermore, the activity, which was called distal splicing factor (DSF), enhanced utilization of an upstream 5' splice site on a simian virus 40 early pre-mRNA, suggesting that the factor acts in a position-dependent, substrate-independent fashion. Several lines of evidence are presented suggesting that DSF is a non-small nuclear ribonucleoprotein protein. Finally, a functional interaction is described between DSF and ASF, a protein that enhances use of downstream 5' splice sites.

L12 ANSWER 44 OF 45 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 35
ACCESSION NUMBER: 91208655 EMBASE
DOCUMENT NUMBER: 1991208655
TITLE: Structure of the human MDR3 gene and physical mapping of the human MDR locus.
AUTHOR: Lincke C.R.; Smit J.J.M.; Van der Velde-Koerts T.; Borst P.
CORPORATE SOURCE: Division of Molecular Biology, The Netherlands Cancer Inst., Plesmanlaan 121,1066 CX Amsterdam, Netherlands
SOURCE: Journal of Biological Chemistry, (1991) 266/8 (5303-5310). ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Two genes, MDR1 and MDR3, constitute the human P-glycoprotein gene family. [illegible] evolutionary relationship between the three known classes of mammalian P-glycoprotein genes, we have cloned the MDR3 gene and compared its structure with that of the human MDR1 and the mouse mdr1 (mdr1b) genes analyzed by other groups. The MDR3 gene contains 28 exons and 27 of these contain coding sequences for the two homologous halves of the protein that correlate with functional domains. This structure is virtually identical to that of the human MDR1 gene and the mouse mdr1 (mdr1b) gene, indicating that the exon/intron structure was fixed before the duplication events that generated different classes of P-glycoproteins, but after the P-glycoproteins diverged from related genes, like the **cystic fibrosis** transmembrane conductance regulator (CFTR) gene, which has an entirely different exon/intron structure. The four alternatively spliced transcripts of the MDR3 gene arise from **alternative splicing** of exons 23 and 26. Our analysis of DNA clones covering about 120 kilobases (kb) of the human MDR locus, including the entire MDR3 gene (74 kb) and the intergenic region between both genes (34 kb), combined with pulsed-field gel electrophoresis data shows that the human MDR locus covers about 230 kb. In contrast to the mouse mdr genes, both human genes are transcribed

in the same direction (MDR3 located downstream of MDR1). The CpG-rich sequences marking the 5' ends of both genes are hypomethylated to different extents in different cell lines. Hypomethylation roughly correlates with transcriptional activity.

L12 ANSWER 45 OF 45 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 36
ACCESSION NUMBER: 1992:565021 CAPLUS
DOCUMENT NUMBER: 117:165021
TITLE: Primary structure of the human splicing factor ASF reveals similarities with Drosophila regulators
AUTHOR(S): Ge, Hui; Zuo, Ping; Manley, James L.
CORPORATE SOURCE: Dep. Biol. Sci., Columbia Univ., New York, NY, 10027, USA
SOURCE: Cell (Cambridge, Mass.) (1991), 66(2), 373-82
CODEN: CELLB5; ISSN: 0092-8674
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The purifn. of a human protein, called **alternative splicing factor** (ASF), that can switch utilization of alternative 5' splice sites in an SV40 early pre-mRNA was previously described. Here, the isolation of a cDNA, designated ASF-1, that encodes this protein is reported. ASF-1 consists of 248 amino acid residues, including an 80-residue RNA-binding domain at its N-terminus and a 50-residue C-terminal region that is 80% serine plus arginine. ASF-1 produced in Escherichia coli can activate splicing in vitro and switch 5' splice-site utilization, establishing that the recombinant protein is sufficient to supply these activities. Anal. of addnl. cDNAs revealed that ASF pre-mRNA can itself be alternatively spliced, surprisingly, by utilization of a shared 5' splice site and 2 closely spaced 3' splice sites. Use of the upstream site results in a second mRNA (ASF-2) in which translation of the downstream exon occurs extensively in an alternative reading frame distinct from ASF-1.

09/421,891

L3 ANSWER 1 OF 12 MEDLINE
AN 2000007306 MEDLINE
DN 20007306
TI **Aberrant splicing** in the PKD2 gene as a cause of polycystic kidney **disease**.
AU Reynolds D M; Hayashi T; Cai Y; Veldhuisen B; Watnick T J; Lens X M; Mochizuki T; Qian F; Maeda Y; Li L; Fossdal R; Coto E; Wu G; Breuning M H; Germino G G; Peters D J; Somlo S
CS Department of Medicine, Albert Einstein College of Medicine, Bronx, New York, USA.
NC R01DK48383 (NIDDK)
SO JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY, (1999 Nov) 10 (11) 2342-51.
Journal code: A6H. ISSN: 1046-6673.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
EW 20000104
AB It is estimated that approximately 15% of families with autosomal dominant polycystic kidney disease (ADPKD) have mutations in PKD2. Identification of these mutations is central to identifying functionally important regions of gene and to understanding the mechanisms underlying the pathogenesis of the disorder. The current study describes mutations in six type 2 ADPKD families. Two single base substitution mutations discovered in the ORF in exon 14 constitute the most COOH-terminal pathogenic variants described to date. One of these mutations is a nonsense change and the other encodes an apparent missense variant. Reverse transcription-PCR from patient lymphoblast RNA showed that, in addition, both mutations resulted in out-of-frame splice variants by activating cryptic splice sites via different mechanisms. The apparent missense variant produced such a strong splicing signal that the processed transcript from the mutant chromosome did not contain any of the normally spliced, missense product. A third mutation, a nonconservative missense change affecting a negatively charged residue in the third transmembrane span, is likely pathogenic and defines a highly conserved residue consistent with a potential channel subunit function for polycystin-2. The remaining three mutations included two frame shifts resulting from deletion of one or two bases in exons 6 and 10, respectively, and a nonsense mutation due to a single base substitution in exon 4. The study also defined a novel intragenic polymorphism in exon 1 that will be useful in analyzing "second hits" in PKD2. Finally, the study demonstrates that there are reduced levels of normal polycystin-2 protein in lymphoblast lines from PKD2-affected individuals and that truncated mutant polycystin-2 cannot be detected in patient lymphoblasts, suggesting that the latter may be unstable in at least some tissues. The mutations described will serve as critical reagents for future functional studies in PKD2.

CT Check Tags: Human; Support, U.S. Gov't, P.H.S.
*Kidney, Polycystic, Autosomal Dominant: GE, genetics
*Membrane Proteins: GE, genetics
*Mutation
Polymorphism (Genetics)
Reverse Transcriptase Polymerase Chain Reaction
*RNA Splicing
CN 0 (Membrane Proteins); 0 (PKD2 protein)

L3 ANSWER 2 OF 12 MEDLINE
AN 95187166 MEDLINE
DN 95187166
TI **Aberrant splicing** in adult onset glycogen storage **disease** type II (GSDII): molecular identification of an IVS1 (-13T-->G) mutation in a majority of patients and a novel IVS10 (+1GT-->CT) mutation.
AU Huie M L; Chen A S; Tsujino S; Shanske S; DiMauro S; Engel A G; Hirschhorn R
CS New York University Medical Center, Department of Medicine, NY 10016.
SO HUMAN MOLECULAR GENETICS, (1994 Dec) 3 (12) 2231-6.
Journal code: BRC. ISSN: 0964-6906.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199506
AB Two newly identified splice site mutations (IVS1 -13T-->G and IVS10 +1GT-->CT) were found in a patient with adult onset of the autosomal recessive disorder glycogen storage disease type II (GSDII). The IVS1 -13T-->G transversion in the acceptor splice site was found on one allele in over two thirds of adult onset GSDII patients studied (28/41), but was not seen in 58 normal or 12 infantile onset GSDII chromosomes. Molecular analysis of cDNA from the index patient and four additional, ethnically different, individuals carrying the IVS1 -13T-->G transversion showed splicing out of the first coding exon as well as rare utilization of a cryptic splice site in the exon. An IVS10 +1GT-->CT transversion, unique to the index patient, was detected on the second chromosome. The IVS10 +1GT-->CT results in splicing out of exon 10 including part of the enzyme catalytic site. Additionally, a large deletion encompassing exon 18, previously described in four unrelated patients, was also detected in three unrelated adult GSDII patients, two of whom carried the IVS1 -13T-->G transversion. The frequency of the IVS1 splice site mutation suggests that detection of this mutation could potentially aid in the diagnosis of the phenotypically variable syndrome of adult onset GSDII. The finding that the -13T-->G mutation is a very common mutation in adult onset GSDII patients of varying ethnic and racial backgrounds, suggests that it is either an ancient mutation or confers a selective advantage. Although to our knowledge these are the first splice site mutations to be reported for GSDII, additional splice site mutations are likely and could provide the basis for later onset disease in GSDII.
CT Check Tags: Case Report; Female; Human; Support, Non-U.S. Gov't
Adult
Age of Onset
Alleles
Base Sequence
Case-Control Studies
Chromosomes, Human, Pair 17
DNA Transposable Elements: GE, genetics
DNA, Complementary: IP, isolation & purification
*Glycogen Storage Disease Type II: GE, genetics
Molecular Sequence Data
*Mutation
*RNA Splicing

RNA, Messenger: GE, genetics
Sequence Deletion
CN 0 (DNA Transposable Elements); 0 (DNA, Complementary); 0 (RNA, Messenger)
GEN GAA

L3 ANSWER 3 OF 12 MEDLINE
AN 94215899 MEDLINE
DN 94215899
TI Construction of a novel database containing aberrant splicing mutations of
mammalian genes.
AU Nakai K; Sakamoto H
CS National Institute for Basic Biology, Okazaki, Japan..
SO GENE, (1994 Apr 20) 141 (2) 171-7.
Journal code: FOP. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199407
AB To explore the rules for mammalian splice-site selection using a statistical approach, we constructed an **aberrant splicing** database containing an extensive collection of mammalian genetic **disease** mutations (90 genes, 209 mutations). From this database, we confirmed that: (1) more than 90% of mutations either destroy or create the splice-site consensus sequences; (2) the number of mutations mapped at individual residues in the splice-site regions roughly correlates to their conservation degrees in the consensus sequences; (3) about half of the observed aberrant splicing is exon skipping, while intron retention is rarely observed; (4) almost all of the major cryptic sites, activated by mutations, are mapped within an about 100-nt region from the authentic splice sites. Furthermore, we found that: (5) mutations are observed more frequently in the 5' splice-site region than in the 3' splice site region; (6) splice sites that are newly created by mutations are located upstream from the authentic splice sites. Hopefully, these observations will be used as rules for constructing a more effective prediction system of exon sequences.
CT Check Tags: Human; Support, Non-U.S. Gov't
Consensus Sequence
*Databases, Factual
Exons
*Mutation
Point Mutation
*RNA Splicing

L3 ANSWER 5 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:509367 BIOSIS
DN PREV199900509367
TI **Aberrant splicing** in the presenilin-1 intron 4 mutation causes presenile Alzheimers **disease** by increased abeta42 secretion.
AU Cruts, M. (1); De Jonghe, C. (1); Rogaeva, E. A.; Tysoe, C.; Singleton, A.; Vanderstichele, H.; Meschino, W.; Dermaut, B. (1); Vanderhoeven, I. (1); Backhovens, H. (1); Vanmechelen, E.; Morris, C. M.; Hardy, J.; Rubinsztein, D. C.; St. George-Hyslop, P. H.; Van Broeckhoven, C. (1)
CS (1) Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), Department of Biochemistry, University of Antwerp (UIA), Antwerpen Belgium
SO American Journal of Human Genetics, (Oct., 1999) Vol. 65, No. 4, pp. A291.
Meeting Info.: 49th Annual Meeting of the American Society of Human Genetics San Francisco, California, USA October 19-23, 1999 The American Society of Human Genetics
. ISSN: 0002-9297.
DT Conference
LA English
CC Genetics and Cytogenetics - Human *03508
Behavioral Biology - Human Behavior *07004
Biochemical Studies - General *10060
Metabolism - Metabolic Disorders *13020
Psychiatry - Psychopathology; Psychodynamics and Therapy *21002
Developmental Biology - Embryology - General and Descriptive *25502
Nervous System - General; Methods *20501
Biophysics - General Biophysical Studies *10502
General Biology - Symposia, Transactions and Proceedings of Conferences, Congresses, Review Annuals *00520
BC Hominidae 86215
IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics); Neurology (Human Medicine, Medical Sciences); Psychiatry (Human Medicine, Medical
IT Diseases
mendelian genetic disorders: genetic disease, molecular bases; presenile Alzheimer's disease: behavioral and mental disorders, genetics, nervous system disease
IT Chemicals & Biochemicals
presenilin-1; A-beta-42: secretion; human PSEN1 gene (Hominidae): aberrant splicing, intron 4 mutation
IT Miscellaneous Descriptors
Meeting Abstract; Meeting Poster
ORGN Super Taxa
Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
human (Hominidae): patient
ORGN Organism Superterms
Animals; Chordates; Humans; Mammals; Primates; Vertebrates

L3 ANSWER 11 OF 12 TOXLIT
AN 1999:25469 TOXLIT
DN CA-130-348212F
TI Human XLIS gene and product doublecortin and diagnostic and therapeutic uses thereof.
AU Chelly J; Kahn A; Des PV; Pinard J
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AB The present invention relates to the identification of a new gene, called XLIS, and of the XLIS gene product, called doublecortin, as well as to the diagnostic and therapeutic applications of these nucleotide and peptide sequences. The structure of the XLIS gene was unusual in that only 16% of its sequence is coding. Alternative splicing leads to a protein of 360 amino acids (doublecortin) as well as one of 402 amino acids. Missense mutations and a mutation leading to **aberrant splicing** in the XLIS gene were identified which correlated with the **diseases** lissencephaly/agyria-pachygyria and subcortical laminar heterotopia/band heterotopia/dobule cortex syndrome. XLIS transcripts are present at very high levels in fetal brain, esp. in neurons, and are then gradually downregulated and reach an undetectable level in adult brain. The XLIS gene seems to be required for initial steps of neuronal dispersion and cortex lamination.
CC 3-3
ST sequence human gene XLIS doublecortin cDNA; lissencephaly band heterotopia XLIS gene mutatio
RN 225102-64-7; 225102-67-0; 225102-68-1; 225102-70-5; 225102-71-6; 225102-73-8; 225102-74-9; 225102-76-1; 225102-77-2; 225102-79-4; 225102-80-7; 225102-81-8; 225102-83-0; 225102-84-1; 225102-86-3; 225102-88-5; 225102-89-6; 225102-91-0; 225102-93-2; 225102-94-3; 225102-95-4; 225102-97-6; 225102-98-7; 225103-00-4; 225103-01-5; 225103-03-7; 225103-04-8; 225103-05-9; 225103-07-1; 225103-08-2; 225103-10-6; 225103-11-7; 225103-13-9; 225103-14-0; 225103-15-1; 225103-17-3; 225103-18-4; 225103-20-8; 225103-21-9; 225103-22-0; 225103-24-2; 225103-25-3; 202938-39-4; 225103-53-7; 225103-54-8; 225103-27-5; 225103-28-6; 225103-30-0; 225103-31-1; 225103-32-2; 225103-34-4; 225103-36-6; 225103-37-7; 225103-38-8; 225103-40-2; 225103-41-3; 225103-43-5; 225103-44-6; 225103-45-7; 225103-46-8; 225103-47-9; 225103-48-0; 225103-49-1; 225103-50-4; 225103-51-5